EFFECT OF MASTITIS ON THE LIPID FRACTION OF MILK

A Dissertation

by

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ABSTRACT

Effect of Mastitis on the Lipid Fraction of Milk. (December 1972)

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The effect of mastitis on the major lipid components of milk was investigated, using quarter milk samples from individual cows in the University herd. Milks with Wisconsin Mastitis Test (WMT) values < 10 mm were considered negative and those with WMT values > 20 mm positive for mastitis. The average fat globule size was not significantly altered by mastitis; however, the WMT positive milks contained more smaller (< 3.96 μ) fat globules. The WMT positive milks contained higher ($P < 0.001$) lipase activity and acid degree values and had 34.6% ($P < 0.001$) less phospholipids. Concentrations of total fatty acids were 4.0% lower ($P < 0.001$), and free fatty acids were 47.3% higher ($P < 0.001$) in WMT positive milks. The WMT positive samples had higher molar percentages of total fatty acids $C_{4:0}$ to $C_{14:0}$ and lower molar percentages of $C_{16:0}$ and $C_{18:0}$. Molar percentages of the free fatty acids $C_{4:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ were lower, while the molar percentages of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ were higher in WMT positive milks.

The WMT positive milks yielded 10.0% less ($P < 0.05$) milk fat

globule membrane material. Higher ($P < 0.01$) amounts of aldolase and xanthine oxidase were present in the membrane preparation from WMT positive milks. The lipid moiety of the membrane preparation from WMT positive milks contained lower amounts of phospholipid ($P < 0.05$) and higher amounts of cholesterol per gram of membrane. Delipidated, dissociated membrane protein prepared from WMT positive milks was separated into five components by polyacrylamide gel electrophoresis, while the membrane protein prepared from WMT negative milks was separated into three components.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

INTRODUCTION

Milk lipids are composed primarily of mixed triglycerides, mono- and diglycerides, and free fatty acids. These lipids are dispersed in milk as fat droplets enclosed in an extremely thin membrane. This membrane is a lipoprotein complex that stabilizes the oil in water emulsion in milk.

Extensive research has shown that mastitis, an inflammation of the udder, alters the gross composition and physical properties of milk. It has been demonstrated that the fat content is lower in milk from diseased quarters; however, limited information is available concerning the influence of mastitis on the detailed chemical analysis of the major lipid constituents of milk.

The constituents of the lipid phase of milk are derived from blood plasma lipids and by direct synthesis in the mammary gland. The origin of the metabolites for direct synthesis can be traced to the blood. Since the characteristics of mastitic milk show a transition to those of blood and blood is a direct source of the milk fat constituents, it would be expected that mastitis could alter the chemical composition of the lipid components of milk.

Changes in the lipid phase of milk could result in flavor defects and undesirable functional properties, thus causing a reduction in the quality of milk and milk products. The objectives

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The format of this dissertation follows the style of the Journal of Dairy Science.

of this study are (a) to investigate the effect of mastitis on the lipid fraction of milk and (b) to determine the effect of mastitis on the compositional properties of the milk fat globule membrane.

REVIEW OF LITERATURE

This review of literature contains three sections. The first section comprises the information pertaining to the effect of mastitis on the composition and physical properties of milk. The second section reviews the current status of knowledge on milk lipids, and the third section reviews the current literature pertaining to the milk fat globule membrane.

Effect of Mastitis on the Composition and Properties of Milk

Mastitis is a complex process of pathological reactions of the udder tissue in response to physical, chemical, and/or microbiological injury. The mammary gland responds to the injury in the form of inflammation and an alteration in the milk secreted. The economic significance of mastitis is well recognized in terms of production losses and costs of therapy (71). However, the amount of economic losses resulting from changes in compositional and technological properties of mastitic milk is unknown. Although several pathogenic and toxin-producing microorganisms can infect the udder tissue (17), public health problems usually are not encountered. The presence of residual antibiotics used in treating mastitis is low; however, the public health significance of these residuals remains unknown (48).

The nature of the inflammatory process, major causative

microflora, and control measures available have been previously reviewed (16,131,134,148,150). Waes and Van Belleghem (202) discussed mastitis and its effects on milk quality. The purpose of this report is to review the influence of mastitis on the compositional and technological properties of milk.

Detection of Mastitis

A systematic approach to the analysis of the effect of mastitis on milk necessitates consideration of the numerous methods of detecting the disease and the various opinions as to when milk is considered to be mastitic. Mastitis can be detected and confirmed by microbiological diagnosis of the udder microflora (17). It can be detected, also, by measurement of the products formed by inflammation and the alterations in the chemical constituents of milk.

It has been extensively documented that the number of leucocytes in milk is higher when inflammation of the udder occurs. This higher leucocyte level is due primarily to the defensive role of leucocytes against infection. It may occur, however, because of noninfectious factors such as stage of lactation (13,41,150,204), age of the animal (13,150,204), and milking practices (114,129,150). In general, a leucocyte count greater than 500,000 per milliliter in bulk milk is strongly suggestive of mastitis (16).

Various methods are used in estimating the number of leucocytes present in milk. The tedious Direct Microscopic Somatic Cell Count (198) or the rapid Electronic Somatic Cell Count (157) is used in

estimating somatic cells present. Due to the release of such substances as DNA material (28) and catalase (128) from leucocytes, indirect estimates of leucocytes are possible. Such screening tests as the Modified Whiteside Test (198), California Mastitis Test (CMT) (167), Wisconsin Mastitis Test (WMT) (192), and Catalase Test (198) are based on these indirect methods of estimating leucocytes. The results of these methods are altered to varying degrees by factors such as omission of a milking or incomplete milking (114,129,150), presence of antibiotics (13,130), detergents and sanitizers (14,154,155,163), aging of the milk (50,101,114,127, 150,154,155,163,197), temperature history (49,50,101,127,156,163), and/or presence of preservatives (49,50). In a comparative evaluation of these procedures for estimation of leucocytes in milk, the WMT was found to give the best estimation (70,158), while the Electronic Somatic Cell Count is the mose precise (158). Variations in methods of detection and in factors that affect these methods and the undefined initial descriptions of samples cause problems in interpreting and comparing data from various investigators.

Composition of Mastitic Milk

In interpreting compositional data of mastitic milk, one must recognize that the abnormal samples are compared to a "normal" value when, in fact, the milk represented by this value may have been altered by previous or undetectable infection (3). Certain studies using mixed herd bulk milk samples (162,203,211) are acknowledged;

however, the more valuable studies in which individual quarter divided samples were used are emphasized. Because of interference from normally occurring factors other than disease, the use of bulk milk is of limited value in studies on the effect of mastitis on milk composition. Some such factors are genetic, physiological, and managerial. These include breed, stage of lactation, feed, or relative volumes of normal and mastitic milk in the mixture. A comparison of milks obtained from healthy and infected quarters of the same animal is more meaningful because the large variability between individual cows has been removed (3).

Milk represents a complex biological mixture of carbohydrates, lipids, proteins, minerals, and vitamins. The properties of milk are directly related to its composition and dynamic state. Milk constituents are synthesized in part by the secretory cells of the mammary gland and in part transferred from blood via transport mechanisms (107). Since mastitis is believed to alter the selective permeability and to impair the milk synthesizing ability of the secretory cells of the mammary gland, it would be expected that the composition of milk would be altered (164). The characteristics of mastitic milk show a gradual transition to those of blood as the severity of the disease increases (3,110).

Mastitic milk contains lower amounts of total solids (TS) than normal milk. This lower TS is the result of lower amounts of fat and solids-not-fat (SNF) (3,56,129). However, inconsistent TS levels have been obtained when normal and mastitic herd or bulk milks have

been compared (72,162,203). Earlier observations which related extent of compositional changes to the severity of infection have been confirmed by more recent works of Ashworth et al. (3), Natzke et al. (129), and Philpot (147) using quarter divided samples with different CMT scores and Hampton and Randolph (56) using individual quarter samples with different WMT scores. The concentrations of some of the major components of milk are lowered as the severity of the disease increases (Fig. 1). Because not all of the individual

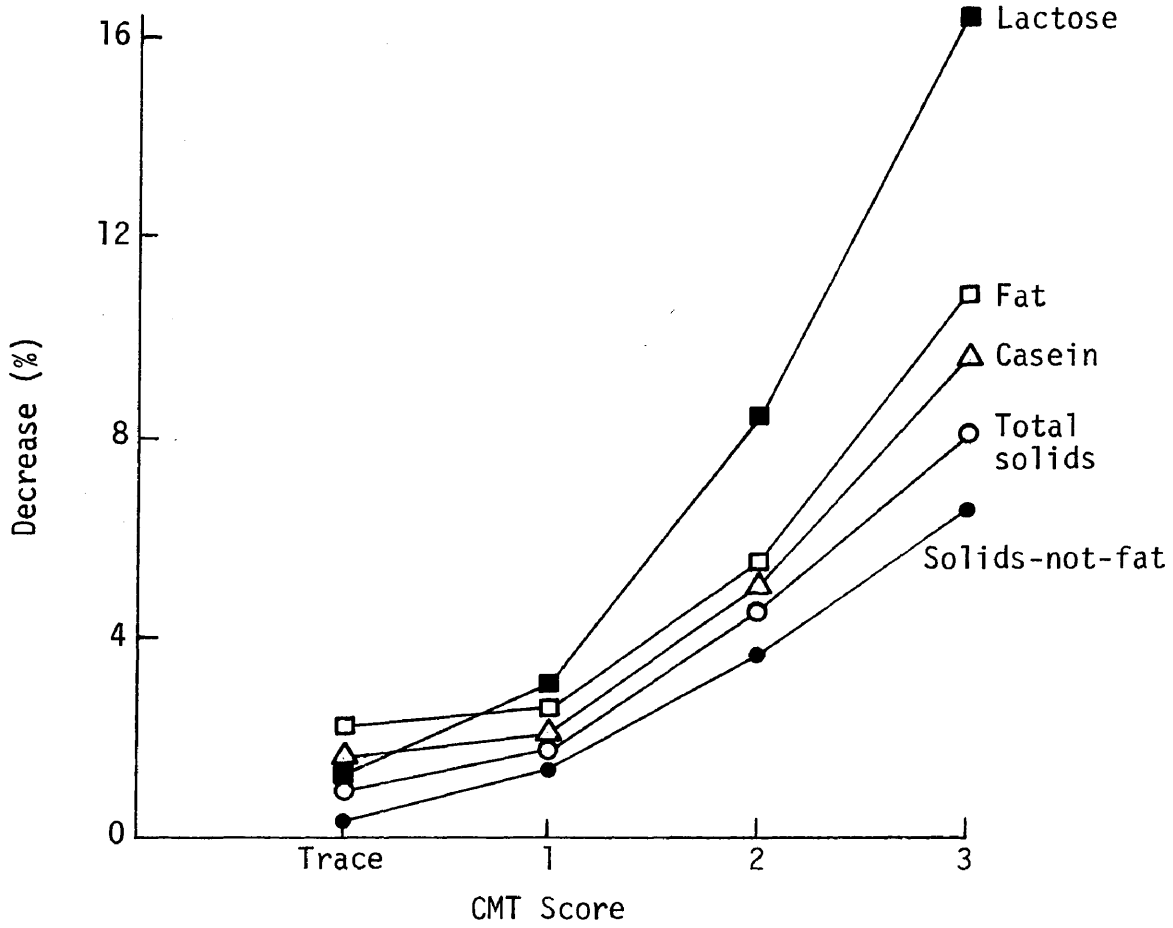

FIG. 1. Relationship between the CMT reaction and gross composition of milk (Data compiled from Ashworth and co-workers (2,3)).

constituents respond to the infection in the same manner, the effect of mastitis on each individual component of milk is discussed.

Milk fat. Abnormal milk contains lower amounts of fat than normal milk (3,56,100,129). In several studies the lower fat level was considered to be statistically significant (3,129). This lower amount of fat was related to the severity of the infection as judged by CMT scores (Fig. 1). While udder inflammation lowers the fat content (Table 1), this decrease also has been detected in unusual conditions such as those which result from the use of fat depressing rations (55) or various types of antibiotic treatments (130). The milk fat content of herd bulk milk samples is not changed significantly by mastitis (72,162,203).

Little is known about the distribution of the various lipid fractions in the fat phase of mastitic milk. Caimi and Redaelli (26) determined that there were significantly lower total and esterified phosphorus fractions in the mastitic than in the normal samples. Kisza et al. (94) reported that cows with mastitis produced lower amounts of phospholipids than healthy cows. Later, Kisza and Batura (92) compared the milk fat from cows with subclinical mastitis with that from normal cows. Mastitic milk contained higher amounts of short ($C_{4:0}$ to $C_{14:0}$) fatty acids and unsaturated fatty acids than normal milk. In these reports the method of detecting the disease was not described. Tolle (196) reported that mastitic milk contained lower amounts of the glycerol esters of the lower fatty acids ($C_{4:0}$ to $C_{12:0}$) while the higher fatty acids, originating from the

TABLE 1. Composition of normal and mastitic milk.

Component	Normal milk		Mastitic milk		% Change	Investigator
	Mean	Range	Mean	Range		
Total solids (%)	13.08		12.01		-8.18	Ashworth et al. (3)
Solids-not-fat (%)	8.90		8.32		-6.52	Ashworth et al. (3)
	8.47	8.06-8.82	8.41	8.06-8.73	-0.71	Hampton and Randolph (56)
Fat (%)	4.16		3.71		-10.82	Ashworth et al. (3)
	3.40	2.80-4.30	3.25	2.60-3.90	-4.41	Hampton and Randolph (56)
Total protein (%)	3.39		3.60		+6.20	Ashworth et al. (3)
	3.34	2.62-3.72	3.43	2.77-3.80	+2.70	Hampton and Randolph (56)
Casein (% of total protein)	75.6		68.3		-9.66	Ashworth et al. (3)
Serum protein (% of total protein)	15.89		17.82		+12.15	Ashworth et al. (3)
Lactose (mg/100 ml)	42.2	36-47	27.2	11-39	-35.60	Cecil (30)

neutral blood lipids were higher because of the increased permeability of the udder.

Proteins. Several investigators have shown that the total protein content of mastitic milk from individual quarters is slightly higher than that of normal milk (Table 1). Ashworth et al. (2), Cecil et al. (30), and Hampton and Randolph (56) compared total protein of normal and mastitic quarter samples by dye-binding techniques. They reported approximately 0.2% higher protein in the mastitic milk samples. This higher total protein content is attributed to the higher serum protein which more than compensates for the lower amounts of casein that occur in mastitic milk.

The use of dye-binding techniques to measure the amount of protein in mastitic milk is thought to lead to erroneous results (52,56,186). Tarassuk et al. (186) reported that a very low correlation coefficient (0.681) was obtained when Kjeldahl and dye-binding procedures were used to determine protein in mastitic milk. This low correlation was considered to be due mainly to the change in the ratio of various protein fractions. Vanderzant and Tennison (200) found that serum proteins had higher dye-binding capacities than did casein. Hampton and Randolph (56) indicated that the variations they observed in the total protein content among WMT positive and negative samples could have been attributed to the differences in dye-binding capacities of the proteins. Recently Waite and Smith (205) reported that mastitis produced variations in the protein values when dye-binding procedures, in contrast to the

Kjeldahl method, were used; however, these minor variations were not considered to be significant.

Caseins. Although the per cent total milk protein varies from animal to animal, the casein-total protein ratio remains relatively constant in milk from healthy cows and is significantly lower due to mastitis. By estimating casein numbers during progressive development of mastitic infection, Vanlandingham et al. (201) observed that the milk composition was affected only after the mammary tissue had reached a certain state of infection. This casein number has, in the past, been suggested as a means of chemical diagnosis of mastitis. Ashworth et al. (3) confirmed these earlier observations by comparing quarter divided CMT samples from the same animal (Fig. 1). Expressed as per cent total protein, casein was lowered by 1.54, 1.99, 4.49, and 6.66% in milks with corresponding CMT scores of Trace (T), 1, 2, and 3.

Limited information is available on the relative proportions of various casein fractions in milk from infected quarters (79,172). Kiddy et al. (79) reported that milk from inflamed quarters contained lower amounts of β- and α-casein. They also reported the occurrence of new, fast migrating unidentified bands on polyacrylamide gels of the mastitic samples. Other investigators have reported similar observations (37,172). Singh and Ganguli (172) and Nagasawa and Tanahashi (126) stated that the amount of α-casein relative to other caseins was higher in mastitic milk than in normal milk.

Sharma and Randolph (70) found that the elution patterns from

Sephadex G-100 of the normal and abnormal skimmilks were similar; however, the centrifugal supernate (56,000 x g for 1 hr) of the mastitic skimmilk contained higher amounts of component I (casein and globulin) and lower amounts of component IV (nonprotein fraction) than normal milk. Sharma and Randolph (171) later studied the effect of mastitis on the composition and functional properties of whole α_s-, β-, and κ-caseins prepared from individual WMT quarter milk samples. While there were no apparent differences in the composition and physical properties of α_s- and β-caseins from normal and abnormal skimmilk, abnormal κ-casein contained higher concentrations of sialic acid and phosphorus. κ-Casein from abnormal skimmilk was less effective in stabilizing α_s- and β-caseins against calcium precipitation, than was κ-casein from normal skimmilk. Some preparations of abnormal κ-casein were completely incapable of stabilizing other caseins. Preparations of κ-casein from abnormal skimmilk deteriorated rapidly during storage of a 1% solution at -18 C, as indicated by the presence of para-κ-casein.

In some concise reports (120,172), it was suggested that the caseins from infected quarters exhibited differences in the hexose and hexosamine content.

Since the caseins are synthesized by the secreting cells, further characterization of the effect of mastitis on these casein fractions may be important to the understanding of casein synthesis in the mammary gland and its unusual functional properties.

Serum proteins. The most extensively studied protein fraction

of milk with respect to how it is affected by mastitis is the serum protein fraction (3,10,27,28,29,55,79,110,196). As discussed earlier, the slightly higher total protein in mastitic milk is due to the higher amounts of serum proteins (Table 1). Ashworth et al. (3) estimated whey protein content of quarter divided samples as per cent total N and reported that samples with CMT levels of T, 1, 2, and 3 contained 0.84, 2.83, 5.59, and 12.15% higher amounts of total N than CMT negative samples. Haenlein et al. (55) reported a positive correlation (+0.45) between WMT and increases in whey protein content. Other investigators (95,96,97,205,211) reported higher amounts of serum proteins in bulk positive samples when compared to bulk negative samples. The higher serum protein level is associated with the altered permeability of the secretory cells. Carrol et al. (29) demonstrated increased permeability of the mammary tissues by obtaining a higher level of blood serum albumin and immunoglobulins within 3 hours after inoculating the lactating quarters with A. *aerogenes*. While the serum albumins declined rapidly to the original levels, the immunoglobulins remained high for the remainder of the lactation period. With the increased transfer into milk of immunoglobulins (100,110,160,172) and blood serum proteins (44,79,110,160,172), there were significantly higher proportions of globulins and changes in the albumin-globulin ratio of the serum proteins. Having separated whey proteins into types and relative amounts using paper electrophoresis, Leece and Legates (110) reported higher proportions of blood-serum albumin and

immunoglobulin in mastitic milk. They suggested that the use of blood-serum albumin would seem to be a reliable indicator of udder infection. In abstracts by Kiddy et al. (79) and Kostov and Dzhurov (100), the higher amount of serum albumin was confirmed. Kiddy et al. (79) reported that mastitis caused relatively lower amounts of α-lactalbumin, while Kostov and Dzhurov (100) reported higher amounts of immunoglobulins and lower amounts of α-lactalbumin and β-lactoglobulin. Other investigators have reported differences between normal and mastitic serum proteins obtained from quarter samples (46,159) and bulk samples (95,96,97,205,211).

Carbohydrates. A lower lactose content appears to be one of the most pronounced effects of mastitis on milk composition (Table 1). For this reason, it has been used in the past as an indicator of udder infection. Ashworth et al. (3) reported that the lactose content of samples with CMT scores of T, 1, 2, and 3 were 1.03, 3.08, 8.35, and 16.32% lower than those of the CMT negative samples (Fig. 1). Walsh and Neave (206) reported that streptococci infections produced lower lactose concentrations than did staphylococci infections. Such differences might be due to the variation in the lactose fermenting ability of these pathogenic strains (150). Mastitic milk also contained lower amounts of lactose in milk when studies were on quarter divided milk samples (30,130, 159,196,207,211) and bulk milk samples (204,212).

The influence of mastitis on the minor carbohydrate constituents of milk has had limited study (30,165,171,172). Cecil et al. (30)

reported that the glycogen level of mastitic milk was approximately eight to nine times higher than normal milk. This was presumably due to the high leucocyte level detected. Rosaschino (165) reported that sialic acid concentration increased from an average of 29.42 mg/100 ml in milk from presumably healthy quarters to 30.75 in milk from chronically infected quarters and to 48.41 in acutely infected quarters. The means by which these degrees of infection were determined was not reported. As mentioned previously, Sharma and Randolph (171) found the sialic acid concentration to be higher in abnormal than in normal κ-casein. More information about the sialic acid content of mastitic milk might contribute to the understanding of the lower rennet activity in mastitic milk.

Minerals. Considerable research has been devoted to clarifying the influence of mastitis on the mineral composition of milk (3,30, 43,94,182). Certain minerals in milk have provided a seemingly accurate indicator of udder infection (3,8,67,201,211). The mineral constituents of milk result in part from the osmotic equilibration of milk and blood (210) and in part from active transport mechanisms. Since the selective permeability is altered during mastitis, it is reasonable to expect the mineral composition to adjust to some new ionic equilibrium.

While several investigators (26,93,94,100,159,196) studied how certain minerals are altered by mastitis, Tallamy and Randolph (182) determined the effects of this disease on the total and free concentrations of the major minerals. The mastitic skimmilks

contained approximately 9, 8, and 4% lower total potassium, inorganic phosphorus, and calcium, respectively, than the corresponding WMT negative samples. These same positive samples contained about 38, 22, 19, 8, and 6% higher sodium, copper, iron, zinc, and magnesium. Similar changes in the relative concentrations of the eight minerals were observed in ultracentrifugates of the positive and negative samples. The positive samples also exhibited higher total and unbound calcium-inorganic phosphorus ratios. The authors (182) suggested that the consistent alteration in the binding level of elements during mastitic infections might reflect abnormal conditions in the udder. This could influence the stability of the caseinate complex (43,171). This alteration in mineral balance could result in unpredictable effects on milk and milk products.

Enzymes. Limited information is available on the effect of mastitis on the levels of enzymes in milk. Mastitic milk exhibits higher catalase (112,128,139,190), lipase (181), phosphatase (125,190), esterase (9,48,112,117), oxidative enzyme (139,190,199), and transaminase (132) activities.

While the higher catalase activity has been used extensively in the past (112,128,198) as a diagnostic aid for mastitis, it is apparent that some of the other enzymes present in milk could be just as valuable (139,181). Tallamy and Randolph (181) reported that lipase activity of WMT positive quarter milks was approximately 7% ($P < 0.01$) higher than the WMT negative milks. Lipase activity was positively correlated ($P < 0.01$) with the WMT as well as the

acid degree values (ADV).

Patterson et al. (136) investigated the effect of mastitis on the oxidative enzymes. They reported that N, N:N', N'-tetramethyl-p-phenylene diamine (TPD-oxidase), catalase, and $NADH_2$-tetrazolium reductase were probably useful in measuring the effect of acute udder infection. Higher levels of these enzymes were present in mastitic milk. Of the enzymes they studied, only catalase had potential as a simple indicator of mastitis.

An elaborate study on the distribution and activity of milk enzymes by Kitchen et al. (98) includes information on mastitic milk. Unfortunately, these data represent bulk milk samples and do not necessarily reveal the actual effect of mastitis on the activity of these enzymes. More information in this area could lead to a determination of the relationship of mastitis to the overall quality of milk and milk products.

Minor components. Comparatively little attention has been directed to the effect of mastitis on the minor components of milk. Hartman and Dryden (58) reviewed the limited material available concerning the effect of mastitis on vitamins. They reported lower amounts of riboflavin and vitamin C and an alteration of other vitamins and associated nutritional factors of milk to some degree when acute mastitic infection was present. However, no sample description was given in terms of methods used to obtain the sample or criteria of infection. This made the comparison of data difficult. An extensive evaluation of the effect of mastitis on vitamins and

other nutritional factors of milk is necessary.

Other minor components such as histamine (30) and lactic acid (149) were higher in mastitic milk than normal milk. The concentration of cystine, glycine, and leucine were lower in mastitic milk when compared to normal milk (36).

Properties of Mastitic Milk

Attempts to utilize mastitic milk for processing and product manufacturing have stimulated studies revealing marked changes in the properties of such milks. Depending on the extent and severity of infection, the changes in milk may be so subtle that the abnormality can be detected only in terms of certain highly sensitive properties of milk such as redox potential (168). Any slight infection, on the other hand, might manifest itself in milk by causing distinct differences in taste, color, appearance, and/or other organoleptic properties. Regardless of the variation in intensity, the differences in properties are not only the result of alterations in the composition and chemical nature of the milk constituents observed so far, but are also due to the variation in their physicochemical state of existence. Some of these differences in properties have only recently been explored with the validity required to reveal the effect of mastitis (Table 2).

Fat globule and casein micelle. The major portions of dairy lipids and caseins exist in milk as particulate structures. Only a few relatively recent reports are available on the effect of mastitis

TABLE 2. Variation in physical properties of milk attributed to mastitis.

Property	Normal milk	Mastitic milk	% change	Investigator
Fat globules				
mean size (μ)	3.305	3.078	-14.6	King (86)
number/mm^3	3774	2860	-24.2	King (86)
Casein micelle				
micellar casein (% of total casein)	87	69	-20.7	Sharma and Randolph (170)
pH	6.64	6.90	+39.2	Erwin et al. (43)
Buffer capacity (dB/dpH)	0.032	0.032	no change	Lane et al. (106)
Redox potential (mv)	324	172	+46.9	Nilsson (133)
Rennet coagulation (min)	30	60	+100	Kiermerier et al. (82)
Curd tension (g)	49	32	-34.8	Tallamy et al. (183)
Curd firmness (g)				
S. lactis C2	81	21	-74.1	Hampton and Randolph (56)
S. cremoris R1	84	17	-79.8	Hampton and Randolph (56)
Acid production (min)				
S. lactis C2	251	263	+0.5	Hampton and Randolph (56)
S. cremoris R1	248	288	+16.1	Hampton and Randolph (56)
Lactic culture inhibition activity (%)	30.0	39.8	+32.7	Randolph (153)
Rancidity (ADV)				
spontaneous	0.57	1.12	+96.5	Tallamy and Randolph (181)
induced	1.03	2.02	+96.1	Tallamy and Randolph (181)

on the physical equilibria of milk in terms of the fat globules and casein micelles.

Using indistinct techniques for diagnosing mastitis, King (86) reported that mastitic milk contained significantly lower amounts of fat globule numbers ($P < 0.001$) and fat globule diameters ($P < 0.05$) (Table 2). Similar results were observed earlier (85,184).

The influence of mastitis on the casein micelle has been overlooked until recently (170,172,185). Sharma and Randolph (170) reported that the concentration of soluble casein was higher and that of micellar casein lower in positive than in negative skimmilk (Table 2). Centrifugation speeds ranging from 56,000 to 144,000 x g for 1 hr resulted in 13 to 15% less casein sedimented in mastitic than in normal skimmilk. After dialysis of WMT positive skimmilks against WMT negative samples, data on the gel filtration of the skimmilks and 56,000 x g supernatants indicated only slight change in the protein sedimentation properties of the skimmilk. Dialysis of normal skimmilks against abnormal skimmilks did not restore the protein sedimentation properties of the abnormal skimmilks to that of the normal skimmilks.

Buffering capacity and pH. Numerous reports (27,30,43,160) have substantiated a higher pH occurs in milk as the degree of infection increases. It has been thought that due to a lower amount of acidic components such as caseins, phosphates, and citrates, a slightly higher pH in mastitic milk could be detected with the pH

indicating dyes used in several mastitis screening tests (3,198). Ashworth et al. (3) reported that pH values of samples with CMT scores of T, 1, 2, and 3 were 0.17, 0.63, 1.78, and 3.85% higher than those of the CMT negative samples.

Lane et al. (106) reported that the buffering capacity of milk is not significantly altered by mastitis. However, a slight shift of the maximum buffering capacity to a more alkaline pH was observed and believed to be explained by factors that cause mastitic milk to be more alkaline than normal milk.

Redox potential. Nilsson (133) reviewed the redox potential of normal and mastitic milk. Individual quarter milk samples from cows having mastitis contained reducing systems different from those in normal milk (Table 2). This reducing property is presumably due to the presence of certain substrates for xanthine oxidase. These substrates are in a concentration sufficiently high to produce the large reduction in potential which was observed. This information provides a basis for the evaluation of the possibility that mastitic milk may have a tendency to become oxidized more readily than normal milk (133).

Rennet coagulation. Rennet coagulation time is longer in mastitic milk than in samples from healthy quarters of the same cow (43,54,56,84). Kiermerier et al. (84) reported that rennet time of CMT positive samples was from 30 to 45-60 min higher than CMT negative samples (Table 2). Other investigators (81,169,174,213) found lower rennet coagulation times for mastitic milk obtained from

bulk milk samples.

Sharma and Randolph (171) studied the properties of isolated bovine caseins. They reported that rennet activity was lower on abnormal than on normal whole casein, but κ-casein from abnormal skimmilk was hydrolyzed by rennet more rapidly than that from normal skimmilk. It is not known whether mastitis affects the enzymatic splitting of κ-casein or the ionic coagulation of the para-κ-casein or both.

The slower rennet clotting of mastitic milk appears to be related to the changes in the chemical composition of milk, particularly to a higher pH, a lower concentration of casein complex, and an altered composition of the protein fractions.

Curd strength. One of the most notable properties of mastitic milk probably is its weak curd formation. This has been a serious problem to cheesemakers and the fermented milk industry. Tallamy et al. (183) reported an inverse relation between the WMT and curd tension of individual quarter milks. The curd tension of the WMT positive samples was approximately 35% lower than that of the corresponding negative samples (Table 2). This effect was more apparent in milks in which the curd tension of the control sample was higher than that in which the normal curd tension was low. Statistically significant ($P < 0.01$) coefficients were obtained when the WMT was correlated to curd tension and to the reduction in curd tension associated with higher leucocyte counts.

Randolph and co-workers (43,56) studied the effects of mastitis on curd firmness. The firmness of the coagulum at pH 5.0 and 4.8 was

more than 70% lower in the positive skimmilk samples than in the corresponding negative samples when single-strain culture of *Streptococcus lactis* C2 and *Streptococcus cremoris* R1 were used (Table 2).

Early investigators (212,213) postulated that the soft curd in mastitic milk is formed as a result of a lower concentration of the caseinate complex rather than as a reduction in the amount of calcium phosphate in the caseins. Erwin et al. (43) demonstrated that, although a significant improvement in the curd tension of the mastitic milk was associated with the equalization of the dialyzable components during the dialysis of mastitic against normal milk, the final values for the abnormal milk were still lower than those of the normal samples. It was suggested that the lowering of the curd tension was related more to the altered nature of the protein than to the total protein concentration. Hampton and Randolph (56) suggested earlier that mastitis might change the casein micelles, thus directly or indirectly influencing curd formation. Changes in the caseins including alteration of the relative proportions of the casein fractions, changes in micelle size, and alteration of properties of the individual caseins were reported recently (79,170, 171). The significance of these changes needs further evaluation in order to clarify the reasons for the lower curd strength. Also, information is needed to determine how the curd forming properties of mastitic milk can be restored to normal milk. The economic losses caused by milk products of lower quality as well as

manufacturing losses should also be investigated.

Heat stability. Studies by Waite and Davis (214) revealed that mastitis produced variations in the coagulation by heat of the caseinate complex. These results were based on herd bulk milk samples. No heat stability values of mastitic milk were established.

Later, Feagan et al. (44,46) reported that mastitic milk produced much lower heat stability values than normal milk. The higher pH in mastitic milk was associated with this low heat stability. Feagan et al. (45) stated that the heat stability of skimmilk powder was influenced by the proportion of mastitic milk present. The natural pH of the normal milk also played an important role in the heat stability values. These data were based on individual quarter milk samples and individual bulk samples in which the Rapid Mastitis Test was used as a detection method. Other investigators (80,95,102) also revealed that mastitic milk provided lower heat stability values. These reports were based on quarter milk samples (80) and bulk milk samples (95,102).

Sharma and Randolph (170) reported that mastitis lowered the heat stability of skimmilk and altered its protein sedimentation properties. Dialysis of mastitic against normal skimmilk improved the heat stability of the mastitic skimmilk. However, dialysis did not completely restore the abnormal values to those of the normal skimmilk values.

Lactic culture activity. The ability of cultures to produce acidity is retarded in mastitic milk. Hampton and Randolph (56)

reported that acid production of *S. lactis* C2 and *S. cremoris* R1 was slower with WMT positive samples than with negative skimmilk samples (Table 2). Culture C2 required 11 to 12 min and Culture R1 38 to 40 min longer to lower the pH of the positive skimmilks to 5.0 and 4.8. The slower rate of culture R1 to produce acid was presumably due to its sensitivity to the natural inhibitory substances normally present in milk. Randolph (153) investigated the influence of mastitis on the inhibitory activity of lactic cultures and the inhibition titers of milk. Inhibitory activity was more apparent in the WMT positive skimmilks than in the negative samples (Table 2). Inhibition titers of the mastitic positive skimmilks for *S. cremoris* R1 and HP were approximately twice those of the mastitic negative samples. It was postulated that certain organisms associated with mastitic infections could possess antigens common to lactic streptococci and that the infectious organisms could initiate an antibody response in the bovine udder. A recent study (203) stated that milk containing high cell counts had no effect on the lactic acid production. However, this study was based on herd bulk milk samples.

Flavor. The total effect of mastitis on the flavor attributes of milk and milk products is unknown. Changes in the composition and properties of milk discussed in the preceding sections may influence the delicate flavor of dairy foods.

Tallamy and Randolph (181) reported that mastitis may contribute to the development of detectable levels of rancid flavor under normal

conditions of milk production and handling. The initial ADV of WMT positive milks were approximately 35 to 40% higher than those of negative samples (Table 2). This relation was observed when lipolysis was spontaneous or induced. The higher ADV was attributed to the higher lipase activity observed in the mastitic milks.

Janzen (72), in a research note on the flavor characteristics of normal and abnormal milk, reported there was an inverse relationship between flavor scores and ADV. The somatic cell concentration had no effect on the flavor of milk. The lack of a correlation between flavor score and somatic cell count possibly could be attributed to the use of herd bulk milk in which there is a blend of various factors in addition to the disease.

Because this organoleptic property is of major importance to milk and milk products, it is imperative that the effect of mastitis on milk and milk product flavors be more thoroughly investigated.

Products. The numerous chemical and physical changes caused by mastitis create considerable difficulties in the manufacture of dairy products such as butter (25,82,180), cheese (23,75,76,82,83, 84,116,161,174), yoghurt (168), and powdered milk (24,45). Most observations of these difficulties are complicated by the combined effects accompanying the use of herd bulk milk. A more accurate evaluation of the effect of mastitis on dairy products will necessitate the use of consistent detection methods and valid controls. Continuous evaluation should be practiced from the initial milk supply, during the processing, and to the final product. This

will provide a basis for economic evaluation of mastitic milk.

Milk Lipids

Milk lipids constitute approximately 3 to 5% of the total composition of milk. These lipids consist essentially of triglycerides together with small proportions of other components including phospholipids, cholesterol, mono- and diglycerides, and free fatty acids. The blood plasma lipids are the major sources of the milk lipids.

The general composition of milk fat has been reviewed by Jack (69) and Kurtz (105), while the physical and chemical properties of the lipid phase of milk have been reviewed by Brunner (19). The biosynthesis of these lipids has been reviewed recently by Lascelles (108). The environmental and physiological factors that influence the fatty acid composition of milk have been reviewed in detail by Garton (51). Since information concerning the influence mastitis has on the lipid components of milk is limited, this review will discuss milk lipids from normal milk.

Composition of Milk Lipids

The constituents of the lipid phase of milk are derived from blood plasma lipids and by direct synthesis in the mammary gland (108, 195). The origin of the metabolites for direct synthesis can be traced back to the blood (108). Therefore, any consideration of milk fat synthesis and composition involves not only the processes of the mammary gland but rumen physiology and the general metabolism of the

cow (115,176).

Bargmann and co-workers (6,7) studied the secretion of fat globules in lactating mice. They reported that aggregation of lipid occurs at the basal region of the secreting cell, and the release of milk fat into the alveolar lumen occurs due to a constricting action. This hypothesis is depicted in Figure 2 which also illustrates the unique plasma membrane surrounding the milk fat globules. The nature and origin of this membrane will be discussed in detail in a later section (Milk Fat Globule Membrane).

The milk fat globules in bovine milk average 2 to 5 μ in diameter. The size distribution of these globules will range from 0.1 to 22 μ in diameter with approximately 95% of them being below 10 μ. The total number of globules present in milk may exceed

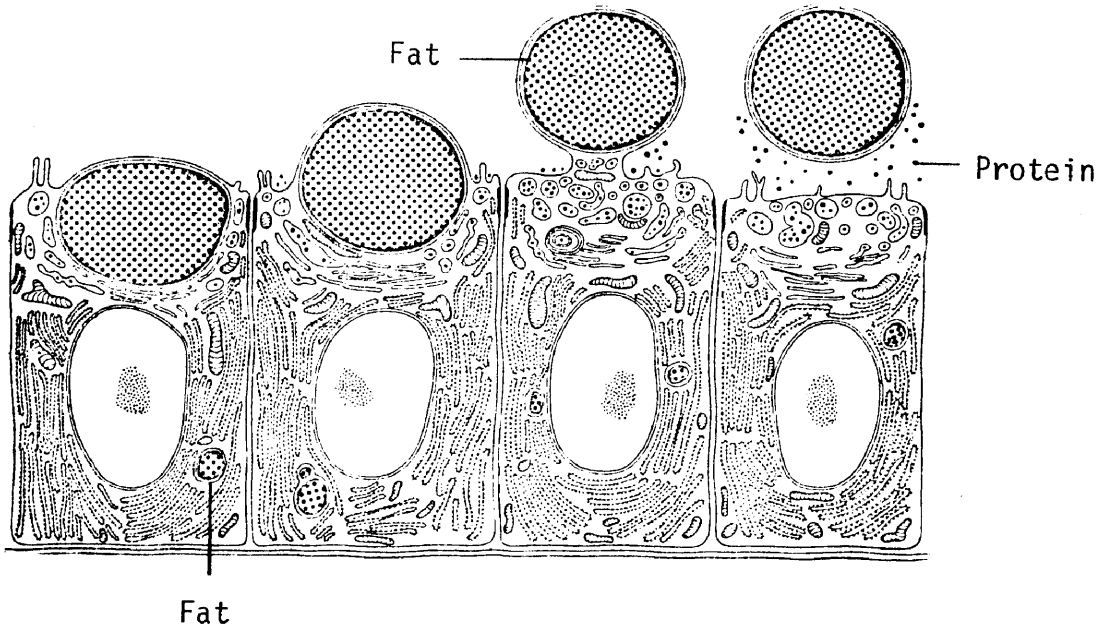

FIG. 2. Secretion of a milk fat droplet as proposed by Bargmann and Knoop.

15 billion per milliliter (38,209). The primary factors involved in the determination of the fat globule size are the amount of membrane material available and the rate of lipid synthesis (19). The fat globule size is determined to certain degrees by such factors as breed of cow, stage of lactation, ration, and presence of mastitis (85,86).

Milk fat is composed of glyceride fractions containing glycerol esters of saturated and unsaturated fatty acids (73,140). The triglyceride fraction comprises approximately 98% of the total lipid composition and is located primarily in the fat globule (105). The major saturated fatty acids present are butyric ($C_{4:0}$), caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), and stearic ($C_{18:0}$). The major unsaturated fatty acids present are decenoic ($C_{10:1}$), palmitoleic ($C_{16:1}$), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$). From the very large number of fatty acids identified (greater than 60) in milk fat, it is evident that there is an exceptionally large possible number of different mixed triglycerides present (73,74,140). Fully saturated glycerides comprise 35% of the milk fat. No simple triglycerides containing three unsaturated fatty acids have been found in milk fat (51,73), though some evidence is available on the presence of traces of tripalmitin in some samples of butterfat (105). Jensen and co-workers (73,74) showed that approximately half of the milk fat composition was made up of glycerides containing at least two unsaturated fatty acid residues.

It has been shown that a definite tendency exists for esterification of fatty acids in specific positions in the triglyceride molecule (15,119,195). The fatty acids $C_{4:0}$ and $C_{6:0}$ are located largely in the primary position; $C_{18:0}$ and $C_{18:1}$ are preferentially in the primary position; $C_{10:0}$, $C_{12:0}$, and $C_{16:0}$ are distributed randomly or with a slight preference for the secondary position; and $C_{14:0}$ is predominantly in the secondary position (15,51,109).

The fatty acids present in milk fat are primarily derived from the lipids of the β-lipoproteins (88,89,217). The mechanism by which the fatty acids $C_{4:0}$ to $C_{16:0}$ are formed is now well established. This mechanism is based on the acetyl-CoA synthetase enzyme complex (107,108,144). Lauryssens (109) reported that the plasma free fatty acids can be rapidly taken up and incorporated into udder glycerides. The glycerol moiety of bovine milk lipids is derived predominantly from blood glucose (115,137) and from the triglycerides of blood lipoproteins (90,195).

After removal of the triglyceride fraction from milk fat, the lipid component remaining consists mainly of phospholipids, cholesterol, mono- and diglycerides, free fatty acids, and the fat soluble vitamins. The principle phospholipids present are choline, ethanolamine, serine and inositol phosphoglycerides, and sphingomyelin (123,216). Milk phospholipids are derived from mammary tissue phospholipids and are very similar to them in composition (216). It is generally accepted that polar lipids such as the phospholipids are associated with proteins in structural membranes

in milk (87).

The amount of cholesterol in milk lipids ranges from 0.25 to 0.40% of the total lipid composition. Approximately one tenth of the cholesterol present is in the ester form (143). Cholesterol originates both from de novo synthesis from acetate within the mammary gland and by transfer from blood into the mammary gland (216). The fatty acid composition of the cholesterol esters is complex, containing high levels of unique fatty acids. These unique fatty acids consist of monosaturated and odd number carbon chains.

The mono- and diglycerides present in milk lipids represent from 0.25 to 0.48% of the total lipid composition. The free fatty acids present represent from 0.10 to 0.44% of the total lipid composition (105). The presence of the mono- and diglycerides as well as the free fatty acids results from either lipolysis or incomplete esterification in the mammary gland (195). The presence of the free fatty acids, particularly the short chain acids, contribute to undesirable flavors in milk (62,91,103).

The fat soluble vitamins A, D, E, and K are constituents of the unsaponifiable material of milk fat. The quantities of these vitamins in milk are largely dependent upon the amounts present in the diet of the animal (58,105).

Importance of Milk Lipids

Milk fat is important to the dairy industry because of economics, nutrition, flavor, and its contributions to the physical properties

of milk and milk products. The most distinctive role of milk lipids is providing flavor for milk products. Many of the compounds responsible for the characteristic flavors are derived from milk lipids. In small amounts, these compounds impart desirable flavors; but in large quantities or in different proportions, they may give rise to off-flavors (62,63,91,103). Flavor volatiles such as lactones and methyl ketones are responsible for the characteristic odors and flavors of milk and milk products (111,122). For example, the $C_{8:0}$, $C_{10:0}$, and $C_{12:0}$ δ-lactones have been found to be the principle contributors to butter flavor (105). Traces (1-2 ppm) of lactones impart an undesirable taste to milk, but lactone levels of 5 ppm in butter contribute a desirable flavor (111). The total lactone content of milk varies with factors such as diet, season, and breed of cow (105).

Milk fat contains 0.03 to 0.45% β-keto glycerides (105). The esterified β-keto acids are hydrolyzed by the presence of water and heat giving rise to methyl ketones. The methyl ketones that have been identified comprise the odd-numbered series from $C_{3:0}$ to $C_{15:0}$ and the short chain acids, $C_{4:0}$, $C_{6:0}$, and $C_{8:0}$ (91). Milk contains approximately 6 ppm of methyl ketones, which is considerably above the methyl ketone flavor threshold value of 1.55 ppm (105).

Lactones and methyl ketones are known to be produced by hydrolytic processes, but free fatty acids are normally liberated by enzymatic hydrolysis. Besides contributing to the natural flavor of milk, the presence of free fatty acids can result in undesirable flavors, such as hydrolytic rancidity. These off-flavors are apparent

when the free short chain fatty acids are present in more than trace amounts (62,63,113,166). As discussed earlier, the enzymatic hydrolysis of milk fat by lipase is increased due to the presence of mastitis (181). Milk fat products are also capable of developing a large number of different oxidative off-flavors (175). These off-flavors have been described as oxidized, cardboard, tallowy, oily, painty, trainy, and fishy (69,175). Increased oxidative off-flavors have occurred due to the increase in the free fatty acid concentrations of milk products (175).

The variability of the milk fat constituents not only affects the flavor of milk products but also changes its functional properties (42,104,136,188,189). Palmer and Tarussuk (136) and Tarussuk and Richardson (189) showed that an increase in the longer chain saturated fatty acids caused an inhibitory effect on the rennet clotting mechanism, whereas the shorter chain fatty acids enhanced this clotting. Rancid cream results in a decrease in churning ability (188) and abnormal foam formation (104). Krukovsky and Sharp (104) concluded that these characteristics were probably due to the increased concentration of fatty acid soaps.

Mono- and diglycerides of milk fat are very important in the reduction of surface tension and improvement of whipping properties of milk and milk products (19). Duthie et al. (42) demonstrated that milk fat obtained from milk subjected to lipolysis produced a lower interfacial tension with water than milk fat obtained from milk which was not subjected to lipolysis. This lower interfacial

tension provided better whipping properties for the milk products.

Milk Fat Globule Membrane

Milk fat is dispersed in milk as spherical fat droplets averaging 2 to 5 μ in diameter (87). The stability of these dispersed fat globules is controlled by colloidal particles oriented at the surface of the fat globules (5,124). These colloidal particles, which are called "membrane" materials, are composed of protein-phospholipid complexes and neutral high-melting glycerides (18). Any chemical or physical modification of this interfacial membrane could result in a loss of stabilizing power and a reduction in the protective action against lipolytic activity (187).

The chemical composition and physical studies of the milk fat globule membrane (MFGM) have been previously reviewed by King (87), Brunner (19), Peereboom (146), and Prentice (152). The membrane accounts for slightly more than 1% of the total weight of the globule and consists of glycerides, phospholipids, cholesterol, a structural-like protein, and various enzymes and metals. There is no information available concerning the influence of mastitis on the properties of the MFGM. Therefore, this material will pertain to the MFGM in normal milk.

Isolation of Membrane Material

In order to isolate the membrane substance for investigation of its properties and composition, it has to be separated from the

components of the aqueous phase of milk (plasma proteins, lactose, and salts) and from the material contained inside the fat globules (fat) (87). There have been many modifications of isolation methods reported (1,4,20,65). In general, the methods for isolating the MFGM involve two steps. The first step is the removal of the aqueous phase from the membrane. It has been found (177) that in order to preserve the organization of the membrane structure, the washing operation should be restricted to the minimum number of steps required to eliminate the plasma proteins. The second major step in the preparation of the MFGM for chemical and physical studies is to remove the fat portion. This is accomplished by destabilization of the fat emulsion by churning the washed cream (178). The MFGM is released into the aqueous phase (buttermilk) during the churning process. It is evident that different methods of isolation could yield membrane materials varying in composition and properties (177). The amount of whole membrane material recovered from 100 g of fat ranges from 0.5 to 1.5 g (177). A general method for isolation of the MFGM is given in Figure 3.

Chemical Composition of the Membrane

There is ample evidence to show that the membrane of milk fat globules is a heterogeneous combination of proteins and lipids which are linked to form a lipoprotein complex approximately 5 to 10 nm in width (19). The protein comprises approximately 40 to 50% of the total membrane content (31). This membrane protein consists of a

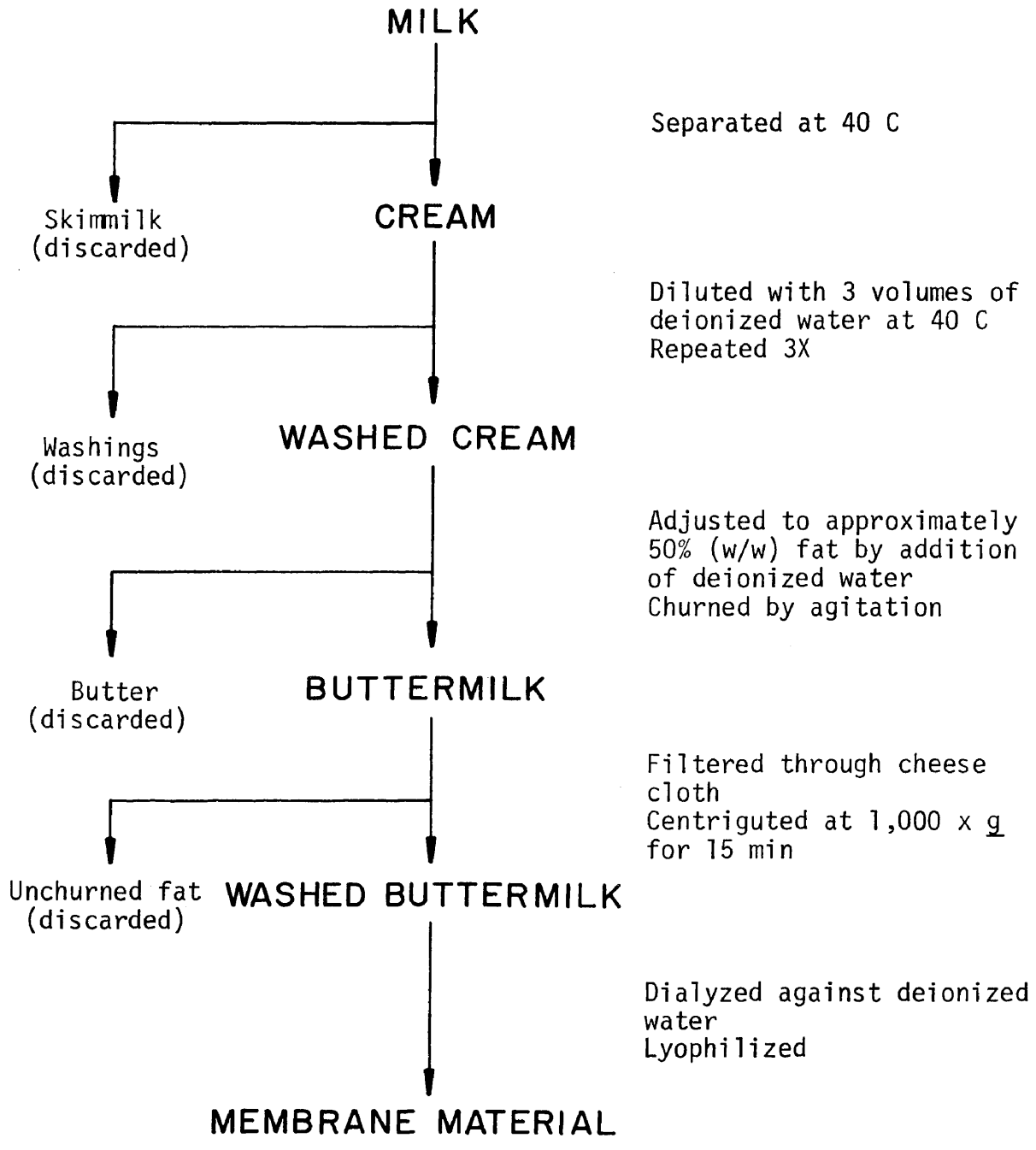

FIG. 3. Procedure for the isolation of milk fat globule membrane material (178).

number of individual proteins, several of which are enzymes (4,40, 145). The interfacial protein mixture has exhibited properties considerably different from those of any of the other milk proteins. The nitrogen content of 12 to 13% is lower, the sulphur content varies greatly, and the phosphorus content is substantially lower than that of the other milk proteins (19). Brunner et al. (20) and Hare et al. (57) reported that the amino acid composition of the membrane protein differs markedly from any of the known milk proteins. There is more arginine, glycine, and phenylalanine and less aspartic acid, glutamic acid, and leucine in the membrane protein than in other milk proteins.

Removal of the lipid fraction from the MFGM results in equal portions of water-soluble and water-insoluble protein fractions (20). The soluble fraction has been classified as a glycoprotein, while the insoluble fraction has been tentatively classified as a pseudokeratin (19,179). Brunner and co-workers (20,21,22) compared the compositional data of the soluble and insoluble fractions. This comparison revealed distinct differences in composition between each fraction and between the other known milk proteins. Brunner (19) suggested, however, before the membrane proteins are accepted as "unique," a more complete study should be made of the minor milk plasma proteins. A recent study by Keenan et al. (77) showed that the purified plasma membrane fraction of lactating bovine mammary secretory tissue was similar in chemical composition with the MFGM fraction isolated from milk. However, the morphology of the two fractions was different. It was suggested that this difference was due to the structural

rearrangement or changes in the lipid-protein ratio.

Electrophoretic patterns of the membrane proteins have been reported (20,21,59,77). The soluble fraction described by Brunner et al. (21,22) showed a single peak by free-boundary electrophoresis at alkaline pH but several peaks at acid pH. Thompson et al. (194) demonstrated that the lipoproteins showed greater electrophoretic mobility than their lipid-free components. They postulated that the glycoproteins exist in various states of association with the lipid moiety. The similarity found in electrophoretic patterns of the various fractions could be the result of incomplete removal of the lipid moiety (19). Keenan et al. (77) subjected the lipid extracted MFGM to polyacrylamide gel electrophoresis. The electrophoretic patterns revealed the presence of several protein components. A major protein was present in the MFGM which was identical with the plasma membrane. The MFGM contained one additional band which was not present in the plasma membrane.

The fact that many of the milk enzymes are associated with the MFGM is well documented (4,40,145). Among the many enzymes associated with the MFGM are xanthine oxidase (4,219), aldolase (151), lipase (187), alkaline phosphatase (219), diaphorase (4), NADH-cytochrome C-reductase and cytochrome (b_5) (4), and ATP-ase (145). Dowben et al. (40) reported the presence of the above enzymes in membrane preparations as well as acid and alkaline phosphomonoesterase, phosphodiesterase, acetyl cholineasterase, and glucose-6-phosphatase. Prentice (152) concluded that the enzymes present in

the MFGM are involved in the deterioration of the milk and seem to have no functional significance once they have left the mammary gland.

The amount of lipid present in the MFGM is dependent upon the method used to obtain the membrane (177). The lipid content can be as high as approximately 65% of the total membrane material (194). Contrary to King's (87) belief, the predominant component of the lipid material has been shown to be the high-melting glyceride fraction (HMG) (12). Patton and Keeney (142) and Thompson et al. (177,193) reported that the HMG contained relatively large quantities of $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$ saturated fatty acids in addition to some $C_{18:1}$ and traces of $C_{18:2}$ and $C_{18:3}$.

The formation of a lipid-lipid type interaction in the MFGM is possible through the bonding of the HMG to the phospholipid present. The major phospholipids associated in this linkage are phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. These phospholipids are present in approximately equal proportions (19,99). The membrane lipids are not restricted to phospholipids and HMG. Other classes of lipids identified with the MFGM are: cholesterol, cholesterol esters, mono- and diglycerides, and free fatty acids (Table 3).

Of the metals associated with the MFGM, copper, molybdenum, and iron have been studied intensively (19). The role of copper as a catalytic agent in oxidative deterioration of phospholipids is well substantiated (146). Approximately one-quarter of the natural copper of milk is bound to the surface of fat globules, probably as a copper-protein complex. This makes the membrane the highest

TABLE 3. Composition of the lipid fraction of the milk fat globule membrane.[a]

Component	Membrane lipids	Whole membrane
	(%)	
Triglycerides	53.41	36.12
Phospholipids	20.35	13.76
Diglycerides	8.14	5.49
Free fatty acids	6.30	4.26
Cholesterol	5.17	3.50
Monoglycerides	4.66	3.14
Cholesterol esters	0.77	0.54
Squalene	0.61	0.40
Carotenoids	0.45	0.30

[a]Data from Thompson et al. (194).

copper-containing protein thus far identified in milk (19,34). Molybdenum and iron are associated almost entirely with the enzyme, xanthine oxidase (40).

Models of the Membrane Complex

The physical structure of the MFGM is not completely understood. However, it is known by the nature of the components of the interfacial membrane that they contribute to the structural characteristics of the lipoprotein complex.

Early proposals of the membrane structure were actually based on only the chemical composition that was known. Mulder (146)

suggested that the fat globule was surrounded by two surface layers with the inner layer originating from the cells of the mammalian gland. The outer layer was formed by the absorption of material from the serum of the milk (Fig. 4). Sommer (173) proposed that the fat droplets were surrounded by a monolayer of phospholipids (Fig. 5). This concept was rapidly dismissed when King (87)

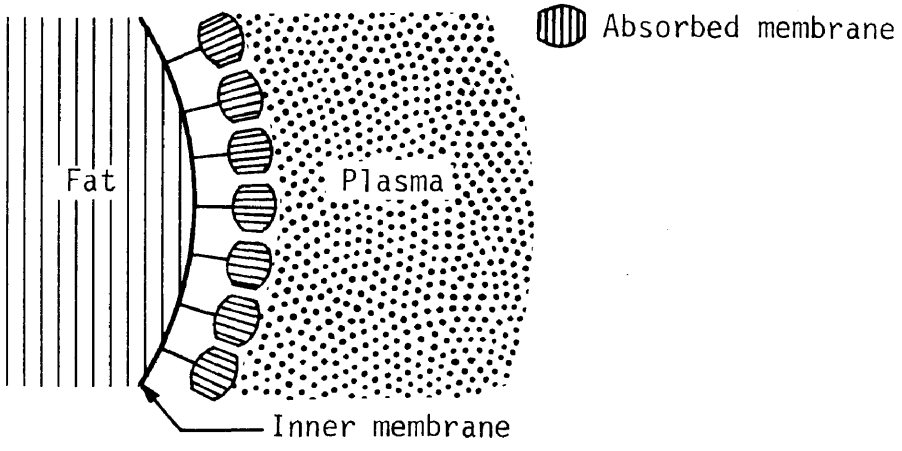

FIG. 4. Model of the milk fat globule membrane according to Mulder (146).

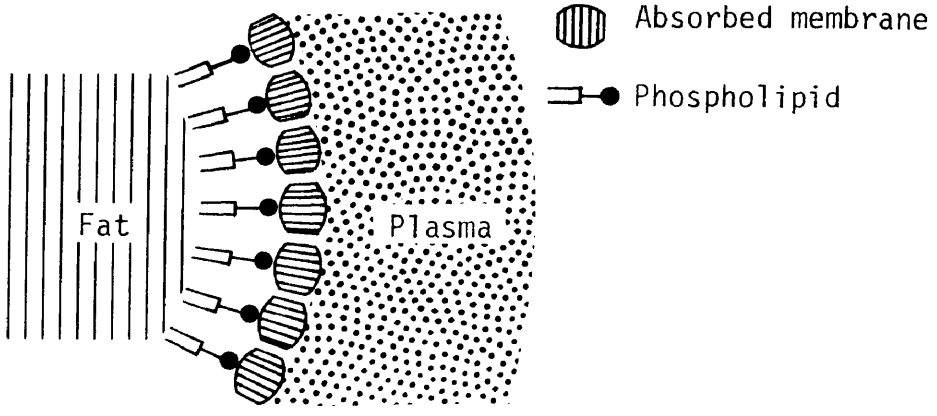

FIG. 5. Model of the milk fat globule membrane according to Sommer (173).

published his excellent review on the MFGM. In this treatise he hypothesized that the membrane consisted of a monolayer consisting of four sublayers: an inner sublayer of high-melting triglyceride molecules; a sublayer of phospholipids with additional molecules like cholesterol and vitamin A; and an outer sublayer consisting of two protein membranes, each of them consisting of a "continuous protein carpet" (Fig. 6).

Morton (124) reported evidence of a lipoprotein complex of the globule membrane which existed as a particulate material in the form of microsomes. He proposed that it was absorbed on a continuous

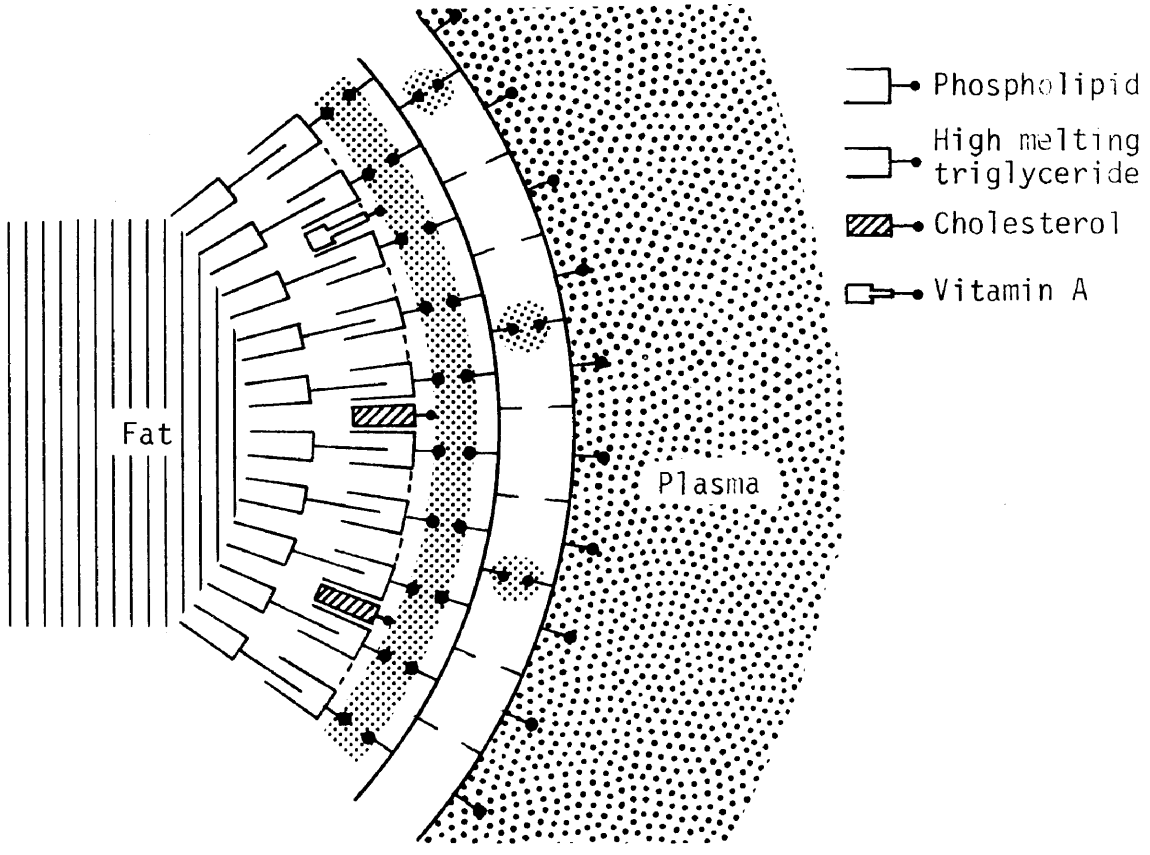

FIG. 6. Model of the milk fat globule membrane as proposed by King (87).

membrane protein layer. The size of the microsomes was estimated to range from 30 to 200 nm. These conclusions were based on studies of electron micrographs of the MFGM. This idea is in clear contrast to the model of King. Morton suggested that the outer layer was a phospholipid-containing layer, while the structure proposed by King had the phospholipid layer inside the two protein layers. Cheeseman and Mabbitt (32) and Peereboom (146) stated that the position of the phospholipids is important in understanding the processes in dairy chemistry. A prime example is the process of copper migration during the manufacture of butter. While the theory of Morton (124) has been supported by some investigators (33,35,60), it has not been accepted by others (40,141,178). By the time Brunner (19) reviewed the information available on the MFGM, there was still no definite choice made between the models of King and Morton.

The physical structure of the MFGM has been further elucidated by electron microscopic studies (64,78,215). Bargmann and co-workers (6,7) observed the secretion of fat globules in lactating rats. They concluded that the milk fat droplets appeared to mature in the apical region of the cell which was progressively enveloped by the plasma membrane and ultimately "pinched" off into the alveolar lumen (Fig. 2, p. 28). It was thought that a second layer of cytoplasmic membrane was added at the moment of secretion.

Dowben et al. (40) proposed that the fat globule interfacial protein is a true biological membrane made up of a triple-layer

structure. They demonstrated this idea with electron micrographs of the structure. Evidence to support this hypothesis was also found in the ability of the fat globule membrane antisera to agglutinate and hemolyze bovine erthrocytes; the permeability of the membrane to potassium; the location of the "unit" membrane structure; and the detection of "microsomal" enzymes. In addition, dense particles were found which may have been identical with the microsomes identified by Morton (124).

The theory of Morton (124) for the physical structure of the MFGM was made more plausible by the investigations of Hayashi and co-workers (60,61). The outer layer of lipoprotein particles, which according to Morton (124) would be absorbed on the inner surface layer, could be readily released by the addition of some type of detergent. Hayashi and co-workers (60,61) succeeded in displacing the outer layer lipoprotein particles by treating the membrane with sodium desoxycholate (DOC). They studied the physicochemical properties of these DOC-released lipoprotein particles in detail. The result showed that the lipoprotein particles were an important part of the globule membrane structure.

The sedimentation patterns of the DOC-released lipoproteins were determined by ultracentrifugation. Most of the particles had a sedimentation coefficient $S_{20,\omega}$ of 13 S. They contained 44% protein and 56% lipids, with a high percentage (76%) of phospholipids in the total lipid fraction. The inner surface layer of the fat globule remained after the release of the lipoprotein particles by

the DOC-treatment. This inner surface layer was composed of about 23% proteins and 77% lipids, with a lower percentage of phospholipids in the total lipid fraction of about 21% (61). Peereboom (146) stated that these results were incompatible with King's model as given in Figure 6.

Based on the above results, Hayashi et al. (60) hypothesized an alternate structural form for the fat globule membrane. In his model, the triglyceride core of the milk fat globule was surrounded by two layers having different physical and chemical properties. The inner membrane layer had a rather insoluble lipid-protein complex which provided a structural matrix on which an outer layer of lipoprotein was associated. The outer membrane layer was absorbed on or associated with the inner layer and had a layer of separate lipoprotein particles. This outer layer could be released by the action of DOC, by which the particles were dispersed as colloidal fat in the aqueous phase. The investigations of Hayashi and co-workers (60,61) were confirmed by Chien and Richardson (33,34, 35) who suggested that the outer layer had at least two different lipoprotein complexes. One of the lipoprotein complexes had compositional and fundamental characteristics similar to the milk microsomes identified by Morton (124). They also stated that the inner membrane layer could have two lipoprotein aggregates, one high in protein and low in lipid and the other fraction low in protein and high in lipid content. After studying the work of Morton (124) and Hayashi (60,61), Peereboom (146) depicted their co-model

schematically (Fig. 7).

It is important to emphasize that the milk fat globules are in equilibrium with the milk serum in which they are suspended (19). Therefore, any change in the ionic concentrations of the serum may be reflected in changes in the MFGM (19).

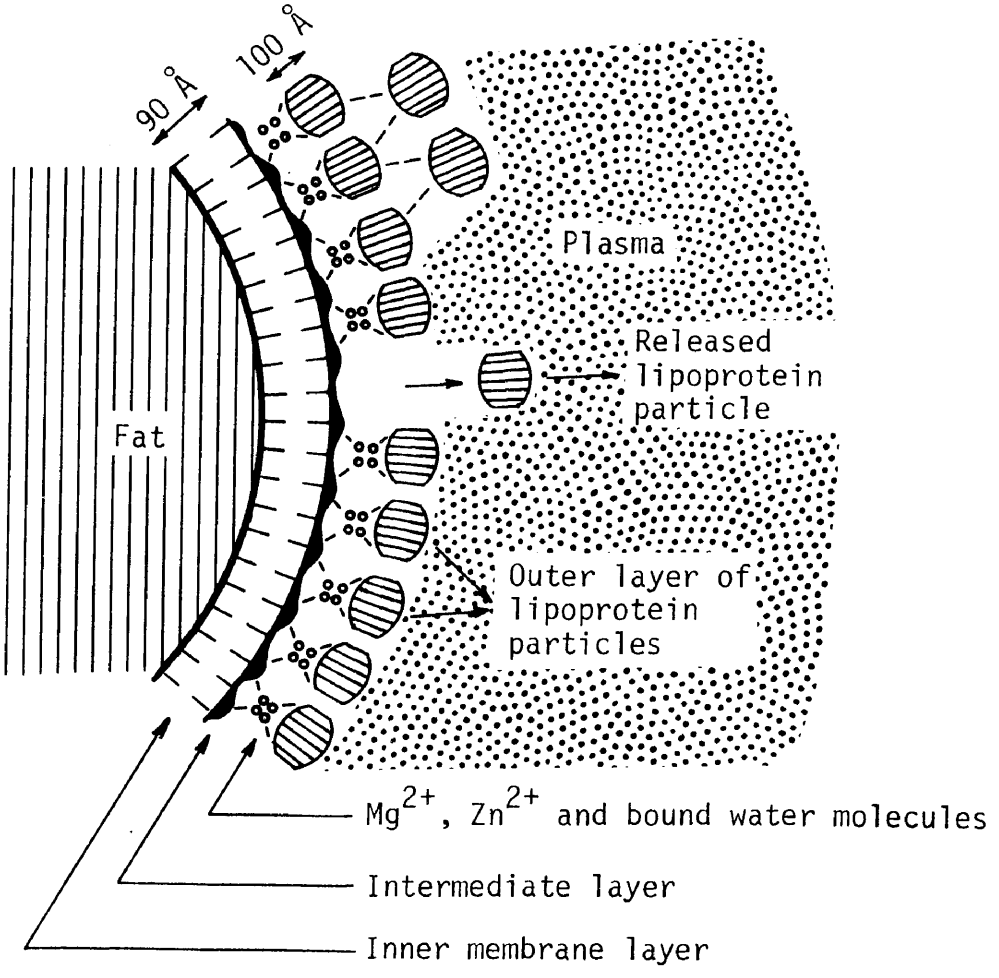

FIG. 7. Morton-Hayashi model of the milk fat globule membrane as viewed by Peereboom (146).

Importance of the Membrane

The MFGM due to its chemical and physical nature is sensitive to the usual agents that tend to disorganize proteins and destroy lipid components. Practically all processing techniques are detrimental to the stability of the membrane material. This includes agitation, heat treatments, homogenization, vacuum concentration, freezing, and drying. The displacement of the MFGM material from the fat globule surface results in various milk products having different fat and phospholipid contents (99). Besides stabilizing the fat globule, the arrangement of the interfacial surface controls the susceptibility of milk to photoactivation, metallic-catalyzed oxidative deterioration, and rancidity (146).

Based on the preceding information, it is evident that mastitis could alter the composition of the lipid fraction of milk. Therefore, the following investigation was undertaken to determine the effect of mastitis on milk lipids and the chemical composition of the membrane surrounding the milk fat globule.

MATERIALS AND METHODS

Selection and Preparation of Samples

Individual quarter milk samples were obtained from the Texas A&M University herd using a quarter-divided milking machine. The samples were cooled in ice water and stored at 4 C until used. Wisconsin Mastitis Test (WMT) values were determined for each sample using the procedure described by Thompson and Postle (192). Samples with WMT values of less than 10 mm were classified as negative, and those with WMT values greater than 20 mm were categorized as positive for mastitis. The fresh individual milks were analyzed for milk fat by the Babcock method (66). Acid degree values were determined by the method of Thomas et al. (191). Approximately 30 ml of each whole milk sample were placed into a glass container (232.03 x 335.03 x 19.05 mm). The container was loosely covered with aluminum foil to prevent contamination, and the samples were frozen at -25 C. Lyopholization was accomplished in 24 to 48 hr at approximately 0.6 mm pressure and less than -10 C plate temperature in a laboratory freeze dryer. The dried sample was removed from the tray and placed in a sterile plastic bag, sealed, and stored at -10 C.

Fat Globule Analysis

Upon completion of milking each cow, an aliquot of milk was immediately diluted (1:50,000) with 1% prefiltered sodium chloride.

The prefiltered diluent was obtained by passing the sodium chloride solution through a filter which excluded < 0.22 μ particles. The fat globule size and distribution was determined in duplicate on each sample using an electronic counting system as described by Walstra et al. (209). Counts were taken on each separate prefiltered diluent prepared to determine the background count of the solution. This background count was subtracted from the counts on the milk dilutions.

Extraction of Lipids

The lipid material of the lyophilized sample was recovered by techniques described by Walstra and Mulder (208). To obtain as complete a recovery of free and bound fatty acids and phospholipids as possible, three extractions were performed. Solvents were removed by a stream of nitrogen (N_2) at 25 C. The lipid was dissolved in hexane and stored until analyzed in sealed tubes at -10 C in the presence of nitrogen.

Phospholipid Analysis

A quantitative aliquot of the lipid material was assayed for phospholipids by the method of Greenbank and Pallansch (53). Lipid phosphorus content was determined by the procedure as described by the AOAC (66). The phospholipid content was determined by multiplying the phosphorus values by a factor of 25.0.

Esterification of Total Fatty Acids

Solvent from each lipid sample was removed by a stream of N_2 at 25 C, and the lipid was quantitatively transferred to a test tube and dissolved in hexane containing a quantitative aliquot of methyl pentadecanote which served as an internal standard. Esterification of total fatty acids to methyl esters was accomplished by the technique of Metcalfe et al. (121) as modified by Dill (39).

Separation and Esterification of Free Fatty Acids

A quantitative aliquot of lipid and internal standard in ether was placed on a silicic acid column prepared by the method of McCarthy and Duthie (118). The separating efficiency of the silicic acid column was determined by placing quantitative mixtures of free fatty acids and triglycerides on the column. Isolation of the free fatty acids was accomplished by the procedure of Iyer et al. (68). Esterification of free fatty acids to methyl esters was accomplished by the procedure of Metcalfe et al. (121) as modified by Dill (39).

Gas Liquid Chromatography Analysis

Methyl esters of total and free fatty acids were analyzed on a Varian-Aerograph model 1200 gas chromatograph equipped with a hydrogen flame ionization detector and a temperature programmer. The column was 1524.00 x 3.43 mm OD stainless steel tubing packed

with 10% EGSS-X on 100-120 mesh Gas Chrom P and was adjusted for on-column injection. Helium was used as the carrier gas. Injector and detector temperatures were 210 and 220 C, respectively. The column oven was programmed as follows: 50 C for 2 min post injection; 50 C to 165 C at 6 degrees/min then held isothermally until the analysis was completed. Peak areas were obtained by triangulation (peak height x width at one-half peak height) and expressed as centimeters squared. Methyl pentadeconate was used for the internal standard to compensate for minor variations in sample size and to determine quantitatively the amounts of acid in each sample. Correction factors for the various fatty acids were obtained to compensate for the esterification and separation techniques and also for the variation due to the relative response of the gas chromatograph to each acid (Appendix Table A-5). All relative response and correction data were related to the internal standard, methyl pentadeconate. At least 3 individual analyses were obtained. Individual fatty acids were identified by retention times of standards subjected to gas chromatography under the same conditions as the samples. All methyl esters were analyzed in triplicate.

Isolation of Fat Globule Membrane

Three groups of individual quarter milk samples from at least five cows having WMT negative and positive milk were obtained. Each group was analyzed separately. Isolation of fat globule membrane

for compositional analysis was accomplished on the uncooled milk using the procedure of Swope and Brunner (178) (Fig. 3, p. 36). Enzymatic assays were conducted on membrane preparations isolated by the procedure of Dowben et al. (40), using a 0.25 M sucrose wash solution containing 2.0×10^{-3} M $MgCl_2$.

Membrane Compositional Assays

Membrane preparations for compositional analysis were dialyzed against deionized water and dried by lyophilization. The lipid moieties of the membrane fractions were extracted with a 2:1 (v/v) mixture of chloroform and methanol (47). Total lipid content of the membrane fractions was estimated from residue weights of aliquots taken from their respective chloroform-methanol extracts. Total protein content of the membrane fractions was estimated by subtracting the total lipid content from 100. The membrane proteins were prepared by repeated extractions (3x) with the above solvent mixture used at the rate of 100 ml/gram of dry membrane material. Cholesterol was determined using the procedure of Bowman and Wolf (11). Phosphorus was determined on the lipoprotein membrane as described by the AOAC (66). Phospholipids were estimated by multiplying phosphorus values by a factor of 25.0. Protein nigrogen content of the extracted membrane proteins was determined by the micro-Kjeldahl procedure (66). Electrophoretic patterns of the delipidated membrane proteins were obtained with a E-C vertical gel electrophoresis apparatus. Electrophoresis was conducted

in polyacrylamide gels containing 7.5% acrylamide in veronal buffer at pH 8.6. Urea (2.5 M) was used as the dissociating agent. After electrophoresis, proteins were made visible by staining with 0.7% Amido Black.

Enzymatic Assays

The following enzymes were assayed on the milk and the membrane preparations essentially as described in the references cited: lipase activity, Parry et al. (138); aldolase, Polis and Shumkler (151); and xanthine oxidase, Zittle et al. (218).

Statistical Analysis

Data was analyzed using the analysis of variance and paired "t" test (135) to determine if mastitis statistically alters the lipid fraction of milk.

The statistical model used to determine if mastitis alters the fat globule size and distribution was as follows:

$$Y_{i,j,k} = \mu + M_i + C_j + MC_{ij} + E_{i,j,k}$$

where

$Y_{i,j,k}$ is the fat globule number at a given size of ith disease of jth cow of kth trial,

μ is the fat globule overall mean number at a given size,

M_i is the departure from the mean caused by ith disease,

C_j is the departure from the mean caused by the jth cow,

MC_{ij} is the departure from the mean caused by the interaction of the ith disease and the jth cow,

$E_{i,j,k}$ is the departure from the mean caused by the unexplained environmental chance effect of test of ith disease of jth cow of kth trial.

The model values were assumed to be random and normally distributed $(0,\sigma^2)$.

$$\sum_i M_i = 0$$

$$\sum_j C_j = 0 \quad .$$

Estimate of actual mean was as follows:

$$E\,[\mu_i] = [(\mu + M_i)\ 50{,}000] \quad .$$

Estimate of actual variance was as follows:

$$E\left[\sigma_i^2\right] = \left[(\sigma^2)\ 50{,}000\right]$$

where

μ and σ^2 are from the model described above.

RESULTS AND DISCUSSION

The effect of mastitis on the lipid fraction of milk was investigated. Wisconsin Mastitis Test negative (WMT < 10 mm) and positive (WMT > 20 mm) quarter milk samples were analyzed for acid degree values (ADV), lipase activity, phospholipid and fat content, fat globule size and distribution, and total and free fatty acid composition. The composition of the milk fat globule membrane (MFGM) isolated from WMT negative and positive milks was also compared.

Effect of Mastitis on Composition and Physical Properties of Milk

The ADV, lipase activity, and phospholipid and fat content were determined on negative and positive individual quarter milk samples (Table 4). The WMT positive milks contained 82.8% and 16.1% higher ADV and lipase activity, respectively. These results are in agreement with the previous report where Tallamy and Randolph (181) reported that mastitis positive samples contained 35 to 40% higher initial ADV and 7% higher lipase activity. They determined that ADV and lipase activity were positively correlated with WMT. This study supports the suggestion of Tallamy and Randolph (181) that mastitis may contribute to the development of detectable levels of rancid flavor in milk.

The WMT positive milks contained 7.2% less fat. Ashworth

TABLE 4. Acid degree values, lipase activity, and phospholipid and fat content of Wisconsin Mastitis Test negative and positive milk samples.[a]

Test	WMT negative	WMT positive
ADV[b]***	0.64 ± 0.05	1.17 ± 0.05
Lipase activity[c]***	1.49 ± 0.05	1.73 ± 0.03
Phospholipid (mg/g fat)***	4.78 ± 0.05	3.55 ± 0.11
Fat[d] (%)***	3.45 ± 0.07	3.20 ± 0.06

[a]Values represent data from ten cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm) from each cow.

[b]ADV represents ml of N KOH required to neutralize 100 ml of fat.

[c]Lipase activity is expressed in microequivalents of 0.025 N base/min/ml of lipase source.

[d]Values represent data from six cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm).

***Values differ significantly ($P < 0.001$). Significance level determined by Paired "t" test.

et al. (3) reported similar results on the effect of mastitis on the fat content of milk (Fig. 1, p. 7). The phospholipid content was approximately 34.6% lower in WMT positive milks. Since phospholipids are important in the protection of fat against lipolysis (105), it is possible that this lower phospholipid content in WMT positive milks may be a contributing factor toward the increase in ADV in WMT positive milks.

One additional sample was obtained in conjunction with the ten paired quarter samples. This sample had a WMT value which was in between (> 10 mm < 20 mm) the negative and positive values.

Lipase activity, ADV, and phospholipid and fat content were determined on this intermediate sample. The values were in between those of the WMT negative and positive samples (Appendix Tables A-1 and A-4). This data suggests that mastitis may affect these tests in a linear fashion.

Fat Globule Analysis

It has been previously reported that an alteration in milk fat is associated with a change in fat globule numbers and diameters (85). King (86) and Tanahashi (184) reported that fat globule numbers and diameters were lower in mastitic milk. Both investigators (87,184) used the microscopic method (209) to determine the average fat globule size and numbers. The microscopic method is tedious and time-consuming and can be a source of possible errors in counting fat globules (38). The use of an electronic particle-sizing and counting device (Coulter Counter) has proven to be a more reliable means of determining the fat globule size distribution and numbers (38). The electronic system was used in this study. The fat globule counts were determined on ten WMT negative and positive individual quarter milk samples. The results are shown in Table 5. Statistical evaluation of the data revealed that the average fat globule size was not significantly altered by mastitis. There was a trend for WMT positive milks to have a lower average fat globule size; however, some positive samples had a higher average fat globule size. These results are not in agreement with previous

TABLE 5. Fat globule size of Wisconsin Mastitis Test negative and positive milks.[a]

Cow	Mean fat globule size	
	WMT negative	WMT positive
	(μ)	
A	3.86 ± 0.66	3.80 ± 0.49
B	3.85 ± 0.98	3.77 ± 0.67
C	3.67 ± 0.64	3.85 ± 0.50
D	3.83 ± 0.76	3.84 ± 0.64
E	4.15 ± 0.78	4.13 ± 0.75
F	4.12 ± 0.73	4.00 ± 0.55
G	3.82 ± 0.56	3.90 ± 0.34
H	4.05 ± 0.51	4.09 ± 0.53
I	4.30 ± 1.08	4.10 ± 0.95
J	4.24 ± 1.00	4.08 ± 0.93
Average	3.99 ± 0.07	3.96 ± 0.04

[a]Samples from individual cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm) from each cow.

reports (86,184) on the effect of mastitis on the average fat globule size. King (86) and Tanahashi (184) reported that the average fat globule diameter of mastitic milk was significantly smaller. The possible explanation for the differences observed in this study as compared to previous reports could be the techniques used in counting the fat globules, the extent of the disease and the type of microorganism causing the disease, and whether the previous reports removed the variable of the cow and the cow-disease interaction.

Using the statistical model described previously (Materials and Methods), values for the fat globule size distribution were

determined (Appendix Table A-3). These values provided data that were independent of variables such as cow and cow-disease interaction. The results are summarized in Tables 6 and 7. Mastitis significantly altered the size distribution of the fat globules. The WMT positive milks contained more fat globules in the size range of 0 to 4.76 μ and lower numbers of fat globules in the size range of 4.76 to 7.14 μ. When considering the overall counting range (0 to 7.14 μ), WMT positive milks contained more fat globules.

The size distribution of fat globules of mastitic milk has not been previously reported. These results reveal that the disease may have some effect on the formation of the fat globule and also on the agglutination properties of these globules. Agglutination could be occurring with the smaller fat globules to form fat globules in the size range of 3 to 4 μ. The higher fat globule numbers in the overall counting range (0 to 7.14 μ) of mastitic milk are in direct conflict with reports by King (86) and Tanahashi (184). The plausible answer for the difference in this study and previous observations must again be placed on the technique used in counting, elimination of known variables, the extent of the disease, and the disease causing microorganisms. Other minor errors may result because the Coulter Counter, when counting particles, does not distinguish between leucocytes (which are high in mastitic milk) and fat globules (209). However, the counting of leucocytes could not account for the large changes that were observed between WMT negative and positive milks.

TABLE 6. Fat globule size distribution of Wisconsin Mastitis Test negative and positive milks.

Fat globule size	Model value[a]	
	$\mu + M_-$[b]	$\mu + M_+$[c]
(μ)		
0 to 2.63***	1168.95	520.10
2.63 to 3.35***	12792.10	2827.25
3.35 to 3.79**	112155.00	118823.00
3.79 to 4.16***	80656.50	97568.40
4.16 to 4.48	19757.30	21000.60
4.48 to 4.76***	4395.30	7268.30
4.76 to 5.25***	9708.85	4736.90
5.25 to 5.68	3271.05	2387.40
5.68 to 6.01**	4386.75	2773.95
6.01 to 6.31***	4977.65	1515.10
6.31 to 6.60	2292.60	1904.40
6.60 to 6.88	3190.10	2759.90
6.88 to 7.14	4170.65	3464.90
0 to 7.14***	262922.00	267550.00

[a]Values represent data from ten cows analyzed by the statistical model $Y_{i,j,k} = \mu + M_i + C_j + MC_{ij} + E_{i,j,k}$.

[b]M_- represents WMT negative.

[c]M_+ represents WMT positive.

**Values differ significantly ($P < 0.01$).

***Values differ significantly ($P < 0.001$).

TABLE 7. Analysis of variance of fat globule size distribution of Wisconsin Mastitis Test negative and positive milks.

Source	df	SS	MS	F
Fat globule size (μ)				
0-2.63				
Mastitis (M)	1	4,210,060.	4,210,060.	402.86***
Cow (C)	9	72,631,400.	8,070,160.	772.24***
Pooled interactions (MC)	9	14,846,400.	1,649,600.	157.85***
Within (W)	20	209,008.	10,450.4	
Total (T)	39	91,896,900.	2,356,330.	
2.63-3.35				
M	1	992,972,000.	992,972,000.	2,681.43***
C	9	3,735,640.000.	415,071,000.	1,120.86***
MC	9	2,365,240,000.	262,805,000.	709.68***
W	20	7,406,300.	370,315.	
T	39	7,101,260,000.	182,084,000.	
3.35-3.79				
M	1	444,662,000.	444,662,000.	11.80**
C	9	31,369,400,000.	3,485,490,000.	92.51***
MC	9	9,131,210,000.	1,014,580,000.	26.93***
W	20	753,503,000.	37,675,200.	
T	39	41,698,800,000.	1,069,200,000.	

TABLE 7 (Continued).

Source	df	SS	MS	F
Fat globule size (μ)				
3.79-4.16				
M	1	2,860,160,000.	2,860,160,000.	99.95***
C	9	109,020,000,000.	12,113,300,000.	423.31***
MC	9	13,476,800,000.	1,497,430,000.	52.33***
W	20	572,308,000.	28,615,400.	
T	39	125,929,000,000.	3,228,950,000.	
4.16-4.48				
M	1	15,457,900.	15,457,900.	0.33
C	9	4,015,500,000.	446,167,000.	9.47***
MC	9	1,323,550,000.	147,061,000.	3.12*
W	20	942,620,000.	47,131,000.	
T	39	6,297,130,000.	161,465,000.	
4.48-4.76				
M	1	82,541,300.	82,541,300.	30.07***
C	9	206,485,000.	22,942,800.	8.36***
MC	9	100,048,000.	11,116,400.	4.05**
W	20	54,891,500.	2,744,580.	
T	39	443,966,000.	11,383,700.	

TABLE 7 (Continued).

Source	df	SS	MS	F
Fat globule size (μ)				
4.76-5.25				
M	1	247,203,000.	247,203,000.	115.43***
C	9	478,490,000.	53,165,500.	24,82***
MC	9	574,104,000.	63,789,300.	29.78***
W	20	42,833,400.	2,141,670.	
T	39	1,342,630,000.	34,426,400.	
5.25-5.68				
M	1	7,808,370.	7,808,370.	3.41
C	9	138,876,000.	15,430,600.	6.73***
MC	9	28,022,500.	3,113,610.	1.36
W	20	45,834,600.	2,291,730.	
T	39	220,541,000.	5,654,910.	
5.68-6.01				
M	1	26,011,200.	26,011,200.	16.71**
C	9	114,821,000.	12,757,900.	8.19***
MC	9	154,252,000.	17,139,200.	11.01***
W	20	31,137,500.	1,556,870.	
T	39	326,222,000.	8,364,670.	

TABLE 7 (Continued).

Source	df	SS	MS	F
Fat globule size (μ)				
6.01-6.31				
M	1	119,893,000.	119,893,000.	53.25***
C	9	379,354,000.	42,150,400.	18.72***
MC	9	189,933,000.	21,103,700.	9.37***
W	20	45,028,000.	2,251,400.	
T	39	734,208,000.	18,825,800.	
6.31-6.60				
M	1	1,506,990.	1,506,990.	3.03
C	9	73,696,500.	8,188,500.	16.45***
MC	9	40,454,900.	4,494,980.	9.03***
W	20	9,957,130.	497,857.	
T	39	125,615,000.	3,220,910.	
6.60-6.88				
M	1	1,850,720.	1,850,720.	0.26
C	9	569,708,000.	63,300,900.	8.98***
MC	9	38,548,100.	4,283,120.	0.61
W	20	140,962,000.	7,048,080.	
T	39	751,068,000.	19,258,200.	

TABLE 7 (Continued).

Source	df	SS	MS	F
Fat globule size (μ)				
6.88-7.14				
M	1	4,980,830.	4,980,830.	0.71
C	9	687,164,000.	76,351,600.	10.90***
MC	9	53,917,200.	5,990,800.	0.86
W	20	140,062,000.	7,003,120.	
T	39	886,125,000.	22,721,100.	
0-7.14				
M	1	214,170,000.	214,170,000.	379.73***
C	9	54,683,500,000.	6,075,940,000.	10,772.97***
MC	9	3,406,120,000.	378,458,000.	671.03***
W	20	11,280,000.	563,999.	
T	39	58,315,000,000.	1,495,260,000.	

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

The higher fat globule numbers in the overall counting range (0 to 7.14 μ) are attributed to the higher numbers of smaller than average (< 3.96 μ) fat globules in WMT positive milks. This higher number of small (< 3.96 μ) fat globules in WMT positive milks would require more phospholipid material to cover the larger surface area. However, WMT positive milks contained less phospholipid material (Table 4, p. 57). This provides further evidence that the fat globules in WMT positive milks are more susceptible to lipolysis and possibly explain the higher ADV.

Effect of Mastitis on the Fatty Acid Composition of Milk Fat

Total Fatty Acid Composition

The average molar percentage distribution of the total fatty acids (TFA) from individual quarter milk samples from ten cows is presented in Table 8. The TFA C_4 to $C_{14:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{18:2}$ were higher and $C_{16:0}$ and $C_{18:0}$ were lower in the WMT positive milks. While the TFA $C_{6:0}$ was approximately 44.0% higher ($P < 0.001$) in WMT positive milks, $C_{16:0}$ and $C_{18:0}$ were approximately 13.3 ($P < 0.008$) and 22.8% ($P < 0.038$) lower, respectively. Molar percentage is a relative value; therefore, a change in one fatty acid must be accompanied by a counter-balancing change in other fatty acids.

Quantitative weighing of aliquots of lipid and the use of an

TABLE 8. Molar percentage of the total fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

Fatty acid	Total fatty acid content		Paired "t" test		
	WMT negative	WMT positive	$\bar{D}$	$S_{\bar{D}}$	α-level
	(molar %)				
4:0	9.56 ± 1.53	10.21 ± 1.63	-0.654	0.587	0.295
6:0	4.36 ± 0.85	6.28 ± 1.17	-1.917	0.364	<0.001
8:0	1.95 ± 0.33	2.14 ± 0.43	-0.188	0.137	0.203
10:0	4.00 ± 0.60	4.62 ± 0.73	-0.624	0.327	0.091
10:1	0.64 ± 0.23	0.73 ± 0.20	-0.089	0.063	0.192
12:0	3.82 ± 0.55	4.27 ± 0.57	-0.443	0.217	0.076
14:0	10.84 ± 1.39	11.15 ± 1.52	-0.308	0.868	>0.400
16:0	25.62 ± 2.64	22.21 ± 2.37	3.403	0.962	0.008
16:1	3.58 ± 0.55	3.65 ± 0.54	-0.075	0.085	0.400
18:0	9.99 ± 0.85	7.71 ± 1.45	2.287	0.895	0.038
18:1	22.71 ± 2.38	24.07 ± 1.61	-1.367	2.080	>0.400
18:2	2.94 ± 0.43	2.96 ± 0.51	-0.025	0.173	>0.400

[a]Values represent data from ten cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm) from each cow.

internal standard (methyl pentadecanote) were used as a means to determine the actual effect of mastitis on the fatty acid composition of milk fat. This value is expressed as standardized weight. The influence of mastitis on the standardized weight of the TFA composition is presented in Table 9. This data reveals the change

TABLE 9. Standardized weight of the total fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

Fatty acid	Total fatty acid content		Paired "t" test		
	WMT negative	WMT positive	$\bar{D}$	$S_{\bar{D}}$	α-level
	(mg/g fat)				
4:0	3.471 ± 0.175	3.639 ± 0.173	0.168	0.071	0.046
6:0	2.086 ± 0.129	2.961 ± 0.184	0.875	0.064	<0.001
8:0	1.162 ± 0.063	1.251 ± 0.081	0.089	0.025	0.008
10:0	2.839 ± 0.134	3.227 ± 0.159	0.388	0.075	<0.001
10:1	0.449 ± 0.050	0.506 ± 0.044	0.057	0.015	0.006
12:0	3.158 ± 0.146	3.464 ± 0.145	0.306	0.060	<0.001
14:0	10.222 ± 0.435	10.328 ± 0.464	0.106	0.270	>0.400
16:0	27.099 ± 0.924	23.098 ± 0.837	4.001	0.354	<0.001
16:1	3.752 ± 0.184	3.761 ± 0.171	0.009	0.041	>0.400
18:0	11.717 ± 0.293	8.870 ± 0.501	2.847	0.309	<0.001
18:1	26.432 ± 0.854	27.541 ± 0.545	1.106	0.774	0.185
18:2	3.398 ± 0.155	3.369 ± 0.182	0.029	0.070	>0.400
Total	95.793 ± 0.416	92.012 ± 0.529	3.781	0.543	<0.001

[a]Values represent data from ten cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm) from each cow.

in each individual fatty acid as related to one gram of fat. All the TFA were significantly ($P < 0.05$) altered except $C_{14:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{18:2}$. The analysis of this data reveals that while some TFA were higher, the overall TFA content was lower ($P < 0.001$)

in WMT positive milks. The short and medium chain TFA $C_{4:0}$ ($P < 0.046$), $C_{6:0}$ ($P < 0.001$), $C_{8:0}$ ($P < 0.008$), $C_{10:0}$ ($P < 0.001$), $C_{10:1}$ ($P < 0.006$), and $C_{12:0}$ ($P < 0.001$) were 4.8, 42.0, 7.6, 13.7, 12.7, and 9.7% higher, respectively, in WMT positive milks. The long chain TFA $C_{16:0}$ ($P < 0.001$) and $C_{18:0}$ ($P < 0.001$) were 14.8 and 24.3% lower in WMT positive milks.

The lower amounts of TFA per gram of fat in WMT positive milks could possibly be the result of decreased metabolic activity in fat metabolism and lipid output. This lower TFA content might also be explained by a decrease in lipid synthesis and possibly an increased utilization of fatty acids for energy which may be required in mastitis infected quarters (164). A possible answer to the alterations in the individual TFA components is also provided by speculation. The constituents of the milk lipids are derived from blood plasma lipids and by direct synthesis in the mammary gland (108,195). The origin of the metabolite for this direct synthesis is the blood (108). Since the characteristics of mastitic milk show a transition to that of blood (3,110), and blood is a direct source of the milk lipid constituents, the milk lipids may be becoming more like the blood lipids. The lower amounts of longer chain fatty acids in the TFA composition could possibly be due to the degradation of these fatty acids. A partial degradation in the long chain fatty acids could also account for the higher amounts of short chain fatty acids observed. The WMT positive milks contained higher amounts of short chain fatty acids which would further support the

suggestion (181) that mastitic milk may contribute to the development of detectable levels of rancid flavor in milk.

Free Fatty Acid Composition

Differences in the free fatty acid (FFA) composition of milk fat from WMT negative and positive milks were indicated by the higher ADV and lipase activity (Table 4, p. 57) found in WMT positive milks. The effect of mastitis on the molar percentage distribution of the FFA composition of milk fat is presented in Table 10. The analyses of this data reveals that only the FFA $C_{6:0}$ and $C_{10:0}$ were significantly ($P < 0.05$) altered by mastitis when comparing relative values.

Determining quantitatively the amount of FFA per gram of fat reveals that mastitis had a highly significant effect on the standardized weight of the FFA composition of milk fat (Table 11). All the FFA determined were higher in the WMT positive milks. Likewise, higher concentrations of total FFA were obtained ($P < 0.001$). The WMT positive milks contained approximately 47.3% higher FFA content. The individual FFA components were 4.7 to 79.5% higher in the WMT positive milks. The concentrations of FFA observed in the WMT negative and positive milks in this study (Table 11) are within the range of the amounts of FFA reported in fresh and rancid milk (91).

The higher concentration of FFA could be attributed to the higher lipase activity present in WMT positive milks (Table 4) and

TABLE 10. Molar percentage of the free fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

Fatty acid	Free fatty acid content		Paired "t" test		
	WMT negative	WMT positive	$\bar{D}$	$S_{\bar{D}}$	α-level
	(molar %)				
4:0	14.39 ± 1.32	12.00 ± 0.71	2.390	1.466	0.145
6:0	4.81 ± 0.40	5.29 ± 0.44	-0.482	0.150	0.012
8:0	3.05 ± 0.18	2.50 ± 0.40	0.554	0.365	0.170
10:0	7.09 ± 0.72	5.18 ± 0.34	1.912	0.729	0.035
10:1	0.89 ± 0.07	1.04 ± 0.09	-0.153	0.089	0.156
12:0	6.33 ± 0.24	5.35 ± 0.54	0.981	0.542	0.105
14:0	9.35 ± 1.18	8.38 ± 0.19	0.972	1.186	0.328
16:0	18.69 ± 0.90	20.28 ± 1.54	-1.590	1.492	0.316
16:1	1.51 ± 0.46	1.95 ± 0.54	-0.437	0.260	0.134
18:0	8.03 ± 1.09	9.96 ± 0.98	-1.930	0.903	0.064
18:1	22.00 ± 1.10	23.78 ± 1.76	-1.781	1.340	0.219
18:2	3.87 ± 0.24	4.30 ± 0.56	-0.435	0.446	0.357

[a]Values represent data from ten cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm) from each cow.

to the increased permeability of the mammary gland to blood lipids (3,110). These data show that the fatty acid constituents of milk fat are significantly altered by mastitis. Previous studies have reported that an alteration in these components cause off-flavor in milk and alter the functional properties of milk products

TABLE 11. Standardized weight of the free fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

Fatty acid	Free fatty acid content		Paired "t" test		
	WMT negative	WMT positive	$\bar{D}$	$S_{\bar{D}}$	α-level
	(mg/g fat)				
4:0	1.364 ± 0.123	1.604 ± 0.075	0.240	0.094	0.036
6:0	0.586 ± 0.049	0.932 ± 0.044	0.346	0.029	<0.001
8:0	0.477 ± 0.032	0.538 ± 0.036	0.061	0.021	0.026
10:0	1.289 ± 0.085	1.350 ± 0.050	0.061	0.066	0.380
10:1	0.163 ± 0.015	0.270 ± 0.014	0.107	0.005	<0.001
12:0	1.349 ± 0.089	1.620 ± 0.084	0.271	0.064	0.004
14:0	2.233 ± 0.130	2.895 ± 0.107	0.662	0.107	<0.001
16:0	5.113 ± 0.374	7.894 ± 0.417	2.781	0.315	<0.001
16:1	0.420 ± 0.055	0.754 ± 0.076	0.334	0.036	<0.001
18:0	2.413 ± 0.173	4.269 ± 0.168	1.856	0.121	<0.001
18:1	6.632 ± 0.507	10.163 ± 0.439	3.531	0.416	<0.001
18:2	1.144 ± 0.078	1.820 ± 0.100	0.676	0.106	<0.001
Total	23.170 ± 1.583	34.117 ± 1.306	10.947	1.054	<0.001

[a]Values represent data from ten cows; one negative (WMT < 10 mm) and one positive (WMT > 20 mm) from each cow.

(42,104,136,188,189). No attempt was made here to detect changes in the organoleptic and physical properties of these milks. However, it is evident that the higher content of FFA in WMT positive milks would contribute to the higher ADV observed. The higher

content of FFA could alter the flavor of milk and milk products. This data further supports the suggestion (181) that mastitis may contribute to the development of detectable levels of rancid flavor in milk.

Effect of Mastitis on the Composition of Milk Fat Globule Membrane

Isolation of Membrane Material

The milk fat globule membrane (MFGM) was isolated from WMT negative and positive milks (Fig. 3, p. 36). During one of the isolation steps (churning of the cream), it was noted that the cream obtained from the two different WMT blends of milk possessed different churning characteristics. The cream from WMT positive milks required notably longer churning time. The butter which was produced after this longer churning time was definitely inferior in structure and texture to that of the WMT negative samples. The plausible explanation for this is that the consistency of butter is dependent to a large extent on the fatty acid composition of the fat phase (19). Also, the fat globule structural characteristics may be altered to such a degree as to affect the churning properties of the cream. The fatty acids of mastitic milk are significantly altered due to mastitis (Tables 9 and 11). These observations are similar to the report of Tarassuk and Palmer (188) who observed that rancid cream was difficult to churn.

Another observation made while isolating the MFGM occurred after freeze drying of the "buttermilk." The membrane material obtained from WMT positive milks was difficult to freeze dry. This appeared to be due to the higher amount of lipid material present. This higher amount of lipid material in the "buttermilk" of WMT positive milks suggests a greater loss of fat in the "buttermilk." This could be related to the fact that WMT positive milks had more smaller ($< 3.96\ \mu$) fat globules which may not be removed by separation. Therefore, it may be speculated that mastitis may cause excessive fat losses in churning and skimmilk powder operations.

Changes in Composition of Membrane

The yield and gross composition of the membrane preparation from WMT negative and positive milk samples are listed in Table 12. The yield of the MFGM was 10.0% less ($P < 0.05$) in the WMT positive milk samples. This difference could be attributed to changes in the physical properties of the cream as well as an actual reduction in the amount of MFGM material. Such factors as water binding properties and temperature dependency of the membrane affect the release of the MFGM into the aqueous phase (177,178). Therefore, any change in the composition of the MFGM may alter its overall yield and physical properties. The lipid content of the membrane prepared from WMT positive milks was 10.0% higher. This is consistent with the observations made during the freeze drying step of the membrane isolation procedure. The membrane prepared from WMT positive milks

TABLE 12. Composition of membrane preparations from Wisconsin Mastitis Test negative and positive milks.[a]

Composition analysis	WMT negative[b]	WMT positive[c]
Total membrane* (g/100 g fat)	1.10 ± 0.02	0.99 ± 0.03
Lipid (% of total membrane)	53.33 ± 0.83	58.70 ± 1.49
Protein (% of total membrane)	46.67 ± 0.83	41.30 ± 1.49

[a]Values represent data from three trials.

[b]WMT negative < 10 mm.

[c]WMT positive > 20 mm.

*Values differ significantly ($P < 0.05$). Significance level determined by Paired "t" test.

contained 11.5% less protein content.

Removal of the lipid moiety from the membrane fraction resulted in a material which was relatively high in protein. The membrane preparation from WMT negative milks consisted of 75.81% protein in comparison with membranes prepared from WMT positive milks which were 72.15% protein. This difference in protein content was not statistically significant. The lipid moiety of the membrane preparation from WMT positive milks contained lower amounts of phospholipid ($P < 0.05$) and higher amounts of cholesterol per gram of membrane (Table 13). Any alteration in the polar lipids of the membrane could result in changes in the MFGM physical properties (19,194). The observed differences in the gross composition of the MFGM from WMT

TABLE 13. Composition of delipidated membrane preparations from Wisconsin Mastitis Test negative and positive milks.[a]

Composition analysis	WMT negative[b]	WMT positive[c]
Protein (%)[d]	75.81 ± 1.54	72.15 ± 3.18
Phospholipid[e]* (mg/gm membrane)	140.75 ± 3.79	130.67 ± 2.72
Cholesterol (mg/gm membrane)	25.40 ± 4.01	28.00 ± 3.56

[a]Values represent data from three trials. Extraction procedure by Folch et al. (47).

[b]WMT negative < 10 mm.

[c]WMT positive > 20 mm.

[d]Protein = N x 6.25.

[e]Phospholipid = P x 25.0.

*Values differ significantly ($P < 0.05$). Significance level determined by Paired "t" test.

negative and positive milks could be due to a decrease in synthesis of the membrane material by the mastitis infected quarters. The changes observed in the composition of MFGM isolated from WMT positive milks could explain the possible variations in the churning characteristics of the cream and the differences in the fat globule distribution.

The MFGM material for enzymatic analysis was isolated by the procedure described by Dowben et al. (40). They reported that the use of their technique for isolation provided preparations with higher enzyme activities than previously reported methods. The WMT

positive whole milk samples contained higher aldolase and lipase activity ($P < 0.01$) (Table 14). Xanthine oxidase activity was found to be 53.4% lower in the WMT positive milks. These results are similar to those previously reported (98).

TABLE 14. Enzyme activity of whole milk and membrane preparations from Wisconsin Mastitis Test negative and positive milks.[a]

Source	Enzyme	WMT negative[b]	WMT positive[c]
Whole milk	Aldolase[d]	0.067 ± 0.009	0.180 ± 0.048
	Lipase[e]**	1.43 ± 0.10	1.71 ± 0.07
	Xanthine oxidase[f]*	11.97 ± 1.33	5.57 ± 0.62
Membrane preparation	Aldolase[d]**	0.010 ± 0.001	0.028 ± 0.002
	Xanthine oxidase[f]**	7.43 ± 0.75	3.38 ± 0.42

[a]Values represent data from three trials.

[b]WMT negative < 10 mm.

[c]WMT positive > 20 mm.

[d]Micromoles of 1, 6 fructose diphosphate split by 1 mg of protein at 37 C in 1 hr.

[e]Microequivalents of 0.025 N base/min/ml of lipase source.

[f]Units of 0.3×10^{-7} M reduced TTC/mg protein.

*Values differ significantly ($P < 0.05$). Significance level determined by Paired "t" test.

**Values differ significantly ($P < 0.01$). Significance level determined by Paired "t" test.

The membrane preparations from WMT negative and positive milks showed similar differences in the enzyme activity that were similar to the enzyme activity differences in the WMT negative and positive milks. However, lipase activity was not detected in any of the membrane preparations. Significantly ($P < 0.01$) higher levels of aldolase and xanthine oxidase activity were present in the membrane preparation from WMT positive milks (Table 14). The presence of these enzymes in the MFGM preparation has been noted as a factor leading to the possibility that the MFGM is a true biological membrane (40). The increase in these enzymes during infection may provide further evidence to support the true biological membrane theory.

Xanthine oxidase activity is known to be dependent on the amount of molybdenum present (218,219). As stated earlier in the literature review, mastitic milk contains lower amounts of molybdenum (67). This lower amount of molybdenum could be the dependent factor for the lower xanthine oxidase activity rather than the actual decrease in the amount of enzyme.

Lipase activity was not detectable in the membrane preparation of WMT negative and positive milks. Tarassuk and Frankel (187) observed lipase activity in MFGM preparations, while others (4,40) have not. Two lipase enzymes have been identified in milk (187). One of these enzymes is associated with the casein fraction of milk and is normally present in milk. The other lipase is thought to be irreversibly absorbed on the MFGM when fresh milk is cooled. However,

it is found in the milk of only a few cows. It must be assumed that the milk obtained for these experiments did not contain any of the latter lipase components.

Polyacrylamide Gel Electrophoresis of Membrane Protein

Mastitis altered the gross composition of the MFGM. This provided a basis to determine if the disease affected the electrophoretic mobility of the membrane protein. The electrophoretic patterns of the chloroform-methanol extracted membrane protein on polyacrylamide gel are illustrated in Figure 8. These electrophoretic patterns were obtained with veronal buffer at pH 8.6 and a dissociating agent (urea, 2.5 M). Preliminary gels had revealed that the membrane protein, without the dissociating agent present, would not separate or migrate. An insoluble protein fraction did not migrate under any conditions.

Membrane protein prepared from WMT negative milks showed distinct bands for components I, IV, and V. It did not reveal components II and III which were present in the membrane protein prepared from WMT positive milks. The differences in the relative component percentages are illustrated in Table 15. The membrane protein prepared from WMT negative milks contained a slow migrating band (component I) which comprised 65.1% of the relative component percentage. This protein also contained two other bands (components IV and V) which were not as distinct in appearance as the first (Fig. 8). The membrane protein prepared from WMT positive milks

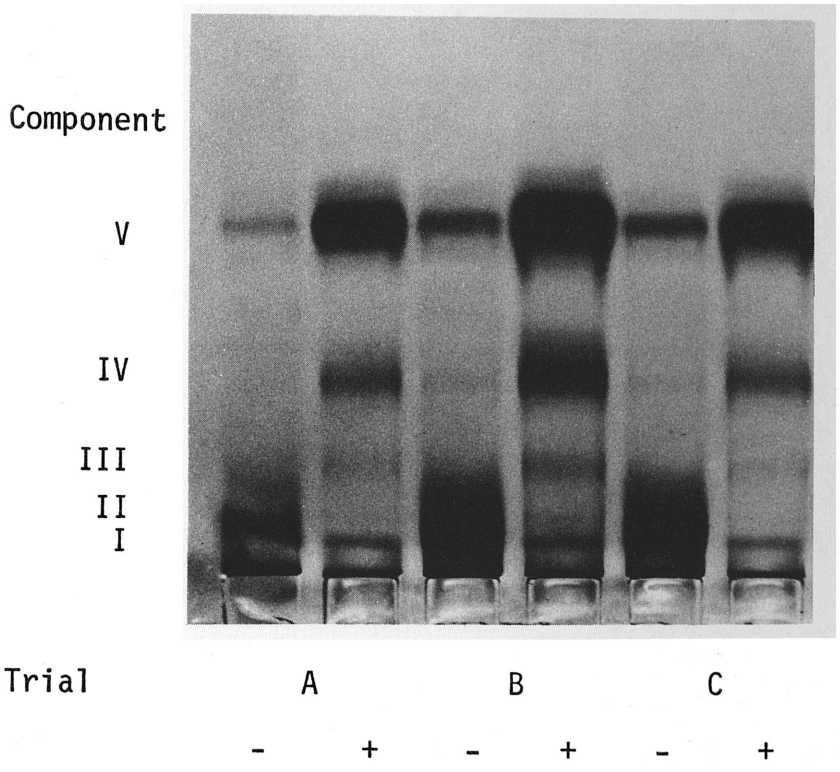

FIG. 8. Polyacrylamide gel electrophoretic patterns of delipidated milk fat globule membrane protein obtained from Wisconsin Mastitis Test negative and positive milks.

TABLE 15. Relative concentration of delipidated milk fat globule membrane protein from Wisconsin Mastitis Test negative and positive milks.[a]

Component	Relative concentration	
	WMT negative[b]	WMT positive[c]
	(%)	
I	65.06 ± 2.74	11.20 ± 1.08
II		1.11 ± 1.11
III		12.47 ± 0.35
IV	5.91 ± 3.00	22.15 ± 0.77
V	29.03 ± 5.74	53.08 ± 0.24

[a]Values represent data from three trials.
[b]WMT negative < 10 mm.
[c]WMT positive > 20 mm.

consisted of five bands (components I-V). The prominent band in these patterns was component V which comprised 53.1% of the relative component percentage. The membrane protein prepared from WMT negative milks contained approximately 82.2% less of component V. Component I was 82.8% lower and component IV was 274.8% higher in the membrane protein prepared from WMT positive milks. Components II and III were not visible in membrane protein prepared from the WMT negative milks. Component II was present only in the Trial B membrane protein prepared from WMT positive milks. However, membrane proteins prepared from WMT negative milks in Trials B and C could

have contained this component, but it was not observed because component I was so prominent. There was no attempt made to identify any of the components.

The observed differences in the electrophoretic patterns of these proteins could be due to the effect of mastitis on the physical structure of the membrane. Harwalker and Brunner (59) showed that the structural organization of the membrane material was dependent primarily on hydrophobic bonding with some covalent disulfide bonds present. If any of these bonds were altered, the electrophoretic patterns could be different. The occurrence of new bands in the membrane prepared from WMT positive milks may be due to incomplete synthesis of the membrane material or degradation of the proteins.

SUMMARY

The effect of mastitis on the individual lipid components of milk and the compositional properties of the milk fat globule membrane was investigated. Acid degree values and lipase activity were higher ($P < 0.001$), and phospholipid and fat content were lower ($P < 0.001$) in WMT positive milks. The average fat globule size was not significantly altered by mastitis; however, the WMT positive milks contained more smaller (< 3.96 μ) fat globules. Concentrations of total fatty acids were lower ($P < 0.001$), and the amounts of free fatty acids were higher ($P < 0.001$) per gram of fat in positive milks. The positive samples had higher molar percentages of total fatty acids $C_{4:0}$ to $C_{14:0}$ and lower molar percentages of $C_{16:0}$ and $C_{18:0}$. Molar percentages of the free fatty acids $C_{4:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ were lower, while the molar percentages of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ were higher in positive milks. The positive milks yielded 10.0% less ($P < 0.05$) milk fat globule membrane material. Membrane material prepared from WMT positive milks contained 10.0% higher concentrations of lipid and 11.5% lower concentrations of protein. Significantly ($P < 0.01$) higher levels of aldolase and xanthine oxidase activity were present in the membrane preparation from WMT positive milks. The lipid moiety of the membrane preparation from WMT positive milks contained lower concentrations of phospholipid ($P < 0.05$) and higher concentrations of cholesterol per gram of membrane. Membrane protein prepared from

WMT positive milks was separated into five components by polyacrylamide gel electrophoresis, while the membrane protein prepared from WMT negative milks was separated into three components.

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APPENDIX

TABLE A-1. Acid degree values, lipase activity, and fat and phospholipid content of Wisconsin Mastitis Test negative and positive milks.

Sample[a]	WMT	ADV[b]	Lipase activity[c]	Fat	Phospholipids
	(mm)			(%)	(mg/g fat)
A	8	0.585	1.62		4.720
	27	1.370	1.78		4.074
B	7	0.515	1.42		4.958
	21	0.965	1.59		4.005
C	8	1.105	1.64		4.643
	36	1.695	1.89		3.447
D	6	0.720	1.51		4.588
	31	1.020	1.63		3.252
E	5	0.420	1.65	3.35	4.775
	31	1.100	1.74	3.20	3.458
F	4	0.480	1.58	3.55	4.986
	17	0.720	1.64	3.45	4.038
	36	1.150	1.69	3.30	2.983
G	4	0.580	1.69	3.50	4.926
	29	0.920	1.86	3.25	3.889
H	4	0.480	1.65	3.45	4.885
	32	0.980	1.90	3.15	3.250
I	9	0.660	1.25	3.85	4.560
	31	1.120	1.56	3.50	3.444
J	4	0.840	0.95	3.05	4.764
	27	1.360	1.58	2.85	3.712

[a]Individual quarter milk samples.
[b]ADV represent ml of N KOH required to neutralize 100 ml of fat.
[c]Lipase activity is expressed in microequivalents of 0.25 N base/min/ml of lipase source.

TABLE A-2. Percentile of the distribution of the test statistic for fat globule size distribution.[a]

Fat globule size	Model value		
	H_m[b]	H_c[c]	H_I[d]
(μ)	(%)		
0 to 2.63	99.95	99.95	99.95
2.63 to 3.35	99.95	99.95	99.95
3.35 to 3.79	99.65	99.95	99.95
3.79 to 4.16	99.95	99.95	99.95
4.16 to 4.48	40.35	99.95	98.47
4.48 to 4.76	99.95	99.95	99.53
4.76 to 5.25	99.95	99.95	99.95
5.25 to 5.68	90.16	99.95	68.19
5.68 to 6.01	99.95	99.95	99.95
6.01 to 6.31	99.95	99.95	99.95
6.31 to 6.60	90.22	99.95	99.95
6.60 to 6.88	35.59	99.95	22.74
6.88 to 7.14	56.41	99.95	42.22
0 to 7.14	99.95	99.95	99.95

[a]Test statistic is the null hypothesis on the statistical model $Y_{i,j,k} = \mu + M_i + C_j + MC_{ij} + E_{i,j,k}$.

[b]$H_m = H : M_- , M_+ = 0$; M_- represents WMT negative; M_+ represents WMT positive.

[c]$H_c = H : C_1 , C_2 \ldots C_{10} = 0$; C_i represents individual cow.

[d]H_I = H :MC interaction = 0 ; MC represents pooled interaction.

TABLE A-3. Fat globule size distribution of Wisconsin Mastitis Test negative and positive milks.

Model value[a]	Fat globule size (μ)				
	0 to 2.63	2.63 to 3.35	3.35 to 3.79	3.79 to 4.16	4.16 to 4.48
μ	844.53	7809.65	115489.00	89112.45	20378.90
M_{-}^{b}	324.43	4982.40	-3334.15	-8456.00	-621.65
M_{+}^{c}	-324.43	-4982.40	3334.15	8456.00	621.65
C_1^d	-494.28	-5731.65	39408.30	-49910.70	-8878.40
C_2	3673.73	15142.35	-1021.70	-80323.70	1029.85
C_3	190.23	19882.10	9610.55	-46496.20	-2603.90
C_4	1051.73	7198.60	8457.80	-30179.20	-11600.15
C_5	-663.53	-5787.15	-32748.45	54368.80	-227.65
C_6	-803.28	-5810.65	-31467.95	76760.80	-3109.90
C_7	-764.28	-5698.15	8237.55	18862.05	-1663.40
C_8	-689.78	-6872.40	51719.70	75684.80	21519.60
C_9	-707.03	-7101.65	23894.30	-1289.95	-9310.90
C_{10}	-793.53	-5221.40	27349.30	-17476.70	14844.85

TABLE A-3 (Right-hand continuation).

Model value	Fat globule size (μ)				
	4.48 to 4.76	4.76 to 5.25	5.25 to 5.68	5.68 to 6.01	6.01 to 6.31
μ	5831.80	7222.88	2829.23	3580.35	3246.38
M_{-}^{b}	-1436.50	2485.98	441.83	806.40	1731.28
M_{+}^{c}	1436.50	-2485.98	-441.83	-806.40	-1731.28
C_1^d	-3202.55	1341.63	-1661.48	-1573.10	-858.38
C_2	-1064.80	6011.88	-1204.23	-1511.10	-1933.88
C_3	238.70	-659.63	-982.98	-762.10	-2389.88
C_4	2130.70	-5669.38	221.53	-104.10	-1631.38
C_5	2434.20	591.38	3282.53	1995.15	3319.63
C_6	-393.30	3810.88	2745.03	599.15	-2198.63
C_7	-4336.30	-761.38	-2327.48	-2892.60	-2818.63
C_8	1630.45	2594.13	1927.53	0.65	-1703.63
C_9	2831.95	-3095.38	-842.98	3025.65	3858.88
C_{10}	-269.05	-4164.13	-1157.48	1222.40	6355.88

TABLE A-3 (Right-hand continuation).

Model value	Fat globule size (μ)			
	6.31 to 6.60	6.60 to 6.88	6.88 to 7.14	0 to 7.14
μ	2098.50	2975.00	3817.78	265236.40
M_-[b]	194.10	215.00	352.88	-2313.93
M_+[c]	-194.10	-215.00	-352.88	2313.93
C_1[d]	459.25	-2794.25	-3576.03	-37471.62
C_2	-389.25	-1323.25	-2180.03	-65094.12
C_3	-1531.00	-2768.25	-3673.53	-31945.87
C_4	-1122.50	-990.50	-2018.53	-34255.37
C_5	-692.25	564.75	1380.73	27818.13
C_6	579.25	-1097.75	-93.28	39520.38
C_7	-1591.50	-2580.50	-2448.78	-783.38
C_8	-363.00	-2512.00	-2880.78	36615.88
C_9	2610.50	9799.00	7246.23	30918.62
C_{10}	1262.00	3702.75	8243.98	34677.35

[a]Model is $Y_{i,j,k} = \mu + M_i + C_j + MC_{i,j} + E_{i,j,k}$.

[b]M_- is WMT negative.

[c]M_+ is WMT positive.

[d]C is individual cow 1 to 10.

TABLE A-4. Fat globule size distribution of Wisconsin Mastitis Test negative, intermediate and positive milks.

Model value[a]	Fat globule size (μ)				
	0 to 2.63	2.63 to 3.35	3.35 to 3.79	3.79 to 4.16	4.16 to 4.48
μ	842.78	7772.32	115104.80	91303.37	20842.07
M_{-}^{b}	326.18	5019.73	-2949.98	-10646.92	-1084.82
M_{+}^{c}	-322.68	-4945.07	3718.32	6265.08	158.48
$M_{\pm}^{d}$	-3.50	74.67	-768.33	4381.84	926.33
C_1^{e}	-494.28	-5731.65	39408.30	-49910.70	-8878.40
C_2	3673.73	15142.35	-1021.70	-80323.70	1029.85
C_3	190.23	19882.10	9610.55	-46496.20	-2603.90
C_4	1051.73	7198.60	8457.80	-30179.20	-11600.15
C_5	-663.53	-5787.15	-32748.45	54368.80	-227.65
C_6	-803.28	-5810.65	-31467.95	76760.80	-3109.90
C_7	-764.28	-5698.15	8237.55	18862.05	-1663.40
C_8	-689.78	-6872.40	-51719.70	75684.80	21519.60
C_9	-707.03	-7101.65	23894.30	-1289.95	-9310.90
C_{10}	-793.53	-5221.40	27349.30	-17476.70	14844.85

TABLE A-4 (Right-hand continuation).

Model value	Fat globule size (μ)				
	4.48 to 4.76	4.76 to 5.25	5.25 to 5.68	5.68 to 6.01	6.01 to 6.31
μ	7247.63	5317.96	4343.81	2789.18	2997.46
M_{-}^{b}	-2852.33	4390.89	-1072.76	1597.57	1980.19
M_{+}^{c}	20.67	-581.06	-1956.41	-15.23	-1482.36
$M_{\pm}^{d}$	3758.00	-3809.83	43029.17	-1582.33	-497.83
C_1^e	-3202.55	1341.63	-1661.48	-1573.10	-858.38
C_2	-1064.80	6011.88	-1204.23	-1511.10	-1933.88
C_3	238.70	-659.63	-982.98	-762.10	-2389.88
C_4	2130.70	-5669.38	221.53	-104.10	-1631.38
C_5	2434.20	591.38	3282.53	1995.15	3319.63
C_6	-393.30	3810.88	2745.03	599.15	-2198.63
C_7	4336.30	-761.38	-2327.48	-2892.60	-2818.63
C_8	1630.45	2594.13	1927.53	0.65	-1703.63
C_9	2831.95	-3095.38	-842.98	3025.65	3858.88
C_{10}	-269.05	4164.13	1157.48	1222.40	6355.88

TABLE A-4 (Right-hand continuation).

Model value	Fat globule size (μ)			
	6.31 to 6.60	6.60 to 6.88	6.88 to 7.14	0 to 7.14
μ	1982.25	3984.25	3274.78	267802.60
M_-[b]	310.35	-794.15	-895.88	-4880.18
M_+[c]	-77.85	-1224.35	-190.13	-252.33
$M_\pm$[d]	-232.50	2018.50	1086.00	5132.50
C_1[e]	459.25	-2794.25	3576.03	-37471.63
C_2	-389.25	-1323.25	-2180.03	-65094.12
C_3	-1531.00	-2768.25	-3673.53	-31945.88
C_4	-1122.50	-990.50	-2018.53	-34255.38
C_5	-692.25	564.75	1380.73	27818.12
C_6	579.25	-1097.75	-93.28	39520.38
C_7	-1591.50	-2580.50	-2448.78	-783.38
C_8	-363.00	-2512.00	-2880.78	36615.87
C_9	2610.50	9799.00	7246.23	30918.63
C_{10}	2040.50	3702.75	8243.68	34677.39

[a]Model is $Y_{i,j,k} = \mu + M_i + C_j + MC_{i,j} + E_{i,j,k}$.

[b]M_- is WMT negative.
[c]M_+ is WMT positive.
[d]$M_\pm$ is WMT intermediate.
[e]C is individual cow 1 to 10.

TABLE A-5. Relative molar response of the gas chromatograph.[a]

Fatty acid[b]	Total fatty acid	Free fatty acid
	——(Relative molar response)——	
4:0	1.089 ± 0.011	1.003 ± 0.023
6:0	1.009 ± 0.006	1.013 ± 0.017
8:0	0.998 ± 0.005	1.012 ± 0.040
10:0	0.976 ± 0.008	1.006 ± 0.102
10:1	1.000	1.000
12:0	0.992 ± 0.009	0.997 ± 0.041
14:0	0.965 ± 0.006	0.983 ± 0.101
15:0	1.000	1.000
16:0	0.979 ± 0.014	0.992 ± 0.034
16:1	1.000	1.000
18:0	1.019 ± 0.015	1.003 ± 0.097
18:1	1.007 ± 0.029	0.989 ± 0.141
18:2	1.078 ± 0.018	1.043 ± 0.061

[a]Values represent data from three trials; internal standard, methyl pentadeconate.

[b]No. of carbon atoms:No. of double bonds.

[c]Values were assumed to be equal to 1.0.

TABLE A-6. Molar percentage of the total fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

Fatty acid[b]	Total fatty acid composition					
	Cow A		Cow B		Cow C	
	WMT negative[c]	WMT positive[c]	WMT negative	WMT positive	WMT negative	WMT positive
	(molar %)					
4:0	10.12 ± 0.14	10.44 ± 0.69	10.71 ± 0.31	11.37 ± 0.09	8.65 ± 0.53	8.83 ± 0.09
6:0	4.36 ± 0.05	5.95 ± 0.24	4.14 ± 0.22	6.35 ± 0.08	3.80 ± 0.19	5.52 ± 0.11
8:0	2.05 ± 0.09	2.24 ± 0.10	1.65 ± 0.09	1.96 ± 0.01	1.75 ± 0.13	1.88 ± 0.33
10:0	3.57 ± 0.02	4.26 ± 0.09	3.54 ± 0.12	3.64 ± 0.03	4.19 ± 0.05	4.82 ± 0.16
10:1	0.51 ± 0.01	0.72 ± 0.04	0.56 ± 0.14	0.70 ± 0.02	0.80 ± 0.06	0.84 ± 0.05
12:0	3.50 ± 0.04	4.29 ± 0.07	3.22 ± 0.12	3.52 ± 0.01	4.04 ± 0.54	4.52 ± 0.18
14:0	10.12 ± 0.08	12.87 ± 0.08	8.94 ± 0.13	9.03 ± 0.04	12.31 ± 0.07	12.45 ± 0.23
16:0	25.04 ± 0.23	20.39 ± 0.58	22.69 ± 0.10	20.30 ± 1.07	27.43 ± 0.55	23.55 ± 0.83
16:1	2.75 ± 0.03	2.82 ± 0.02	4.21 ± 0.09	4.36 ± 0.02	3.29 ± 0.47	3.34 ± 0.11
18:0	10.58 ± 0.03	9.28 ± 0.19	10.18 ± 0.06	8.83 ± 0.04	9.25 ± 1.28	6.68 ± 0.23
18:1	24.19 ± 0.24	23.02 ± 0.27	26.59 ± 0.03	26.34 ± 0.96	21.59 ± 1.33	24.71 ± 0.13
18:2	3.21 ± 0.01	3.72 ± 0.38	3.59 ± 0.07	3.61 ± 0.01	2.90 ± 0.20	2.87 ± 0.22

TABLE A-6 (Right-hand continuation).

Fatty acid	Total fatty acid composition					
	Cow D		Cow E		Cow F	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(molar %)					
4:0	9.02 ± 0.10	9.19 ± 0.21	7.64 ± 0.16	7.78 ± 0.37	9.66 ± 0.26	9.96 ± 0.29
6:0	4.66 ± 0.11	6.64 ± 0.12	5.37 ± 0.08	7.52 ± 0.14	3.88 ± 0.15	5.61 ± 0.10
8:0	2.11 ± 0.04	2.19 ± 0.22	1.82 ± 0.26	1.95 ± 0.04	1.68 ± 0.04	1.81 ± 0.07
10:0	3.81 ± 0.14	4.41 ± 0.17	3.56 ± 0.08	4.39 ± 0.11	4.19 ± 0.10	5.01 ± 0.09
10:1	0.53 ± 0.04	0.59 ± 0.11	0.84 ± 0.07	0.89 ± 0.03	0.59 ± 0.03	0.66 ± 0.03
12:0	3.47 ± 0.19	4.27 ± 0.16	3.90 ± 0.18	4.08 ± 0.02	4.03 ± 0.03	4.29 ± 0.14
14:0	11.80 ± 0.07	11.92 ± 0.38	12.84 ± 0.67	13.00 ± 0.03	10.43 ± 0.10	10.39 ± 0.13
16:0	29.36 ± 1.16	26.40 ± 0.62	27.05 ± 0.86	22.57 ± 0.55	27.82 ± 0.59	23.08 ± 0.44
16:1	3.40 ± 0.17	3.41 ± 0.04	3.38 ± 0.14	3.39 ± 0.04	3.64 ± 0.09	3.66 ± 0.06
18:0	8.76 ± 0.12	5.90 ± 0.07	10.06 ± 0.43	6.56 ± 0.07	9.36 ± 0.32	6.57 ± 0.15
18:1	20.51 ± 0.62	22.52 ± 0.42	20.24 ± 0.72	24.58 ± 0.50	22.03 ± 0.24	26.29 ± 0.59
18:2	2.58 ± 0.01	2.57 ± 0.04	3.31 ± 0.05	3.27 ± 0.04	2.68 ± 0.03	2.66 ± 0.03

TABLE A-6 (Right-hand continuation).

Fatty acid	Total fatty acid composition					
	Cow G		Cow H		Cow I	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(molar %)					
4:0	12.96 ± 0.92	13.72 ± 0.15	9.83 ± 0.22	10.53 ± 0.39	7.96 ± 0.47	9.35 ± 0.20
6:0	2.44 ± 0.49	3.62 ± 0.09	4.91 ± 0.08	7.25 ± 0.31	5.01 ± 0.47	7.02 ± 0.09
8:0	2.06 ± 0.04	2.31 ± 0.04	1.90 ± 0.25	1.97 ± 0.09	1.74 ± 0.32	1.81 ± 0.06
10:0	3.08 ± 0.16	3.60 ± 0.07	4.23 ± 0.19	5.07 ± 0.20	4.93 ± 0.11	6.04 ± 0.03
10:1	0.33 ± 0.09	0.39 ± 0.02	0.95 ± 0.02	0.97 ± 0.02	0.95 ± 0.06	1.02 ± 0.18
12:0	3.23 ± 0.17	3.63 ± 0.05	3.47 ± 0.02	3.87 ± 0.06	4.47 ± 0.09	4.72 ± 0.18
14:0	9.22 ± 1.38	9.33 ± 0.10	10.67 ± 0.25	10.37 ± 0.64	9.78 ± 0.10	9.87 ± 0.50
16:0	22.76 ± 2.46	20.65 ± 0.32	27.61 ± 0.57	24.77 ± 8.44	24.90 ± 0.61	22.00 ± 0.88
16:1	4.36 ± 0.16	4.42 ± 0.06	3.64 ± 0.03	3.71 ± 0.12	2.88 ± 0.06	3.16 ± 0.12
18:0	11.63 ± 0.17	10.53 ± 0.97	10.82 ± 0.09	7.27 ± 0.81	9.76 ± 0.29	7.80 ± 0.09
18:1	24.57 ± 2.06	24.47 ± 0.24	19.16 ± 0.70	21.41 ± 0.94	24.91 ± 1.16	24.53 ± 1.21
18:2	3.37 ± 0.21	3.33 ± 0.08	2.83 ± 0.03	2.82 ± 0.01	2.74 ± 0.02	2.69 ± 0.02

TABLE A-6 (Right-hand continuation).

	Total fatty acid composition	
	Cow J	
Fatty acid	WMT negative	WMT positive
	(molar %)	
4:0	8.99 ± 0.26	10.91 ± 0.08
6:0	5.01 ± 0.10	7.29 ± 0.39
8:0	2.76 ± 0.20	3.27 ± 0.04
10:0	4.90 ± 0.03	4.98 ± 0.09
10:1	0.38 ± 0.04	0.53 ± 0.10
12:0	4.91 ± 0.20	5.48 ± 0.21
14:0	12.34 ± 0.25	12.31 ± 0.44
16:0	21.52 ± 0.80	18.43 ± 1.46
16:1	4.22 ± 0.28	4.23 ± 0.05
18:0	9.53 ± 0.09	7.62 ± 1.80
18:1	23.28 ± 1.22	22.85 ± 3.51
18:2	2.18 ± 0.02	2.12 ± 0.08

[a]Values represent data from three trials.

[b]No. of carbon atoms:No. of double bonds.

[c]WMT negative < 10 mm. WMT positive > 20 mm.

TABLE A-7. Standardized weight of the total fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

	Total fatty acid composition					
	Cow A		Cow B		Cow C	
Fatty acid[b]	WMT negative[c]	WMT positive[c]	WMT negative	WMT positive	WMT negative	WMT positive
	(mg/g fat)					
4:0	3.65 ± 0.067	3.74 ± 0.159	3.86 ± 0.050	4.11 ± 0.017	3.16 ± 0.133	3.11 ± 0.032
6:0	2.07 ± 0.024	2.81 ± 0.051	1.97 ± 0.073	3.02 ± 0.035	1.83 ± 0.050	2.57 ± 0.017
8:0	1.21 ± 0.024	1.32 ± 0.041	0.97 ± 0.038	1.16 ± 0.003	1.05 ± 0.051	1.09 ± 0.112
10:0	2.52 ± 0.041	2.99 ± 0.049	2.50 ± 0.068	2.57 ± 0.009	2.99 ± 0.029	3.32 ± 0.079
10:1	0.35 ± 0.033	0.50 ± 0.017	0.39 ± 0.058	0.49 ± 0.009	0.57 ± 0.026	0.57 ± 0.020
12:0	2.87 ± 0.033	3.50 ± 0.054	2.64 ± 0.046	2.89 ± 0.009	3.35 ± 0.239	3.62 ± 0.092
14:0	9.45 ± 0.047	11.95 ± 0.055	8.36 ± 0.150	8.45 ± 0.029	11.66 ± 0.050	11.38 ± 0.139
16:0	26.25 ± 0.391	21.26 ± 0.418	23.83 ± 0.166	21.34 ± 0.733	29.17 ± 0.390	24.18 ± 0.478
16:1	2.86 ± 0.024	2.92 ± 0.029	4.38 ± 0.050	4.54 ± 0.015	3.47 ± 0.291	3.41 ± 0.084
18:0	12.31 ± 0.133	10.74 ± 0.065	11.87 ± 0.070	10.30 ± 0.029	10.90 ± 0.806	7.61 ± 0.176
18:1	27.92 ± 0.118	26.45 ± 0.142	30.76 ± 0.282	30.48 ± 0.532	25.30 ± 1.013	27.95 ± 0.255
18:2	3.68 ± 0.024	4.24 ± 0.229	4.13 ± 0.079	4.14 ± 0.009	3.37 ± 0.154	3.22 ± 0.148
Total	95.14 ± 0.861	92.41 ± 0.446	95.67 ± 0.908	93.48 ± 0.365	96.83 ± 0.572	92.05 ± 0.774

TABLE A-7 (Right-hand continuation).

Fatty acid	Total fatty acid composition					
	Cow D		Cow E		Cow F	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(mg/g fat)					
4:0	3.36 ± 0.037	3.35 ± 0.051	2.79 ± 0.033	2.82 ± 0.082	3.51 ± 0.044	3.51 ± 0.043
6:0	2.29 ± 0.015	3.19 ± 0.044	2.58 ± 0.022	3.59 ± 0.040	1.86 ± 0.040	2.61 ± 0.040
8:0	1.28 ± 0.009	1.31 ± 0.072	1.09 ± 0.090	1.16 ± 0.010	1.00 ± 0.019	1.04 ± 0.029
10:0	2.77 ± 0.043	3.15 ± 0.067	2.54 ± 0.030	3.11 ± 0.046	2.98 ± 0.032	3.46 ± 0.087
10:1	0.38 ± 0.015	0.42 ± 0.050	0.59 ± 0.029	0.62 ± 0.015	0.41 ± 0.009	0.45 ± 0.015
12:0	2.94 ± 0.083	3.54 ± 0.090	3.24 ± 0.088	3.36 ± 0.012	3.33 ± 0.025	3.45 ± 0.111
14:0	11.39 ± 0.086	11.26 ± 0.168	12.16 ± 0.357	12.21 ± 0.030	9.83 ± 0.075	9.51 ± 0.180
16:0	31.84 ± 0.927	28.01 ± 0.520	28.77 ± 0.551	23.81 ± 0.286	29.45 ± 0.413	23.72 ± 0.516
16:1	3.65 ± 0.102	3.58 ± 0.04	3.57 ± 0.087	3.55 ± 0.020	3.83 ± 0.049	3.73 ± 0.078
18:0	10.53 ± 0.026	6.94 ± 0.59	11.87 ± 0.299	7.68 ± 0.052	11.00 ± 0.232	7.49 ± 0.129
18:1	24.48 ± 0.277	26.31 ± 0.147	23.71 ± 0.467	28.56 ± 0.381	25.68 ± 0.105	29.75 ± 0.475
18:2	3.06 ± 0.019	2.98 ± 0.029	3.85 ± 0.032	3.78 ± 0.017	3.10 ± 0.027	2.99 ± 0.064
Total	97.99 ± 0.647	94.03 ± 0.478	96.78 ± 0.091	94.24 ± 0.180	95.99 ± 0.408	91.71 ± 1.429

TABLE A-7 (Right-hand continuation).

Fatty acid	Total fatty acid composition					
	Cow G		Cow H		Cow I	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(mg/g fat)					
4:0	4.73 ± 0.243	4.74 ± 0.087	3.50 ± 0.035	3.82 ± 0.900	2.87 ± 0.096	3.30 ± 0.049
6:0	1.17 ± 0.125	1.65 ± 0.030	2.30 ± 0.150	3.47 ± 0.092	2.38 ± 0.132	3.27 ± 0.017
8:0	1.23 ± 0.009	1.31 ± 0.029	1.11 ± 0.080	1.17 ± 0.025	1.03 ± 0.109	1.04 ± 0.017
10:0	2.19 ± 0.090	2.44 ± 0.044	2.94 ± 0.083	3.59 ± 0.090	3.47 ± 0.046	4.17 ± 0.003
10:1	0.23 ± 0.031	0.26 ± 0.009	0.65 ± 0.006	0.68 ± 0.009	0.66 ± 0.023	0.70 ± 0.077
12:0	2.67 ± 0.078	2.85 ± 0.062	2.81 ± 0.006	3.19 ± 0.025	3.66 ± 0.039	3.79 ± 0.096
14:0	8.72 ± 0.852	8.36 ± 0.153	9.85 ± 0.165	9.74 ± 0.367	9.14 ± 0.066	9.03 ± 0.238
16:0	24.12 ± 1.465	20.78 ± 0.440	28.61 ± 0.252	26.13 ± 0.308	26.13 ± 0.403	22.60 ± 0.450
16:1	4.58 ± 0.059	4.42 ± 0.088	3.74 ± 0.015	3.88 ± 0.071	3.00 ± 0.040	3.22 ± 0.061
18:0	13.67 ± 0.266	11.74 ± 0.468	12.44 ± 0.058	8.51 ± 0.527	11.36 ± 0.207	8.90 ± 0.074
18:1	28.66 ± 1.204	27.12 ± 0.505	21.87 ± 0.529	24.87 ± 0.604	28.79 ± 0.736	27.77 ± 0.868
18:2	3.91 ± 0.140	3.66 ± 0.088	3.21 ± 0.030	3.25 ± 0.013	3.15 ± 0.010	3.02 ± 0.007
Total	95.87 ± 0.762	89.32 ± 1.057	93.04 ± 0.459	92.30 ± 0.375	95.63 ± 0.058	90.80 ± 0.350

TABLE A-7 (Right-hand continuation).

Fatty acid	Total fatty acid composition	
	Cow J	
	WMT negative	WMT positive
	(mg/g fat)	
4:0	3.28 ± 0.042	3.89 ± 0.019
6:0	2.41 ± 0.047	3.43 ± 0.106
8:0	1.65 ± 0.082	1.91 ± 0.015
10:0	3.49 ± 0.035	3.47 ± 0.037
10:1	0.26 ± 0.019	0.37 ± 0.040
12:0	4.07 ± 0.122	4.45 ± 0.096
14:0	11.66 ± 0.069	11.39 ± 0.240
16:0	22.82 ± 0.649	19.15 ± 0.882
16:1	4.44 ± 0.198	4.36 ± 0.032
18:0	11.22 ± 0.124	8.79 ± 1.204
18:1	27.18 ± 0.651	26.15 ± 2.310
18:2	2.52 ± 0.012	2.41 ± 0.055
Total	94.99 ± 0.549	89.78 ± 0.095

[a]Values represent data from three trials.
[b]No. of carbon atoms:No. of double bonds.
[c]WMT negative (-) < 10 mm.
WMT positive (+) > 20 mm.

TABLE A-8. Molar percentage of the free fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

Fatty acid[b]	Free fatty acid composition					
	Cow A		Cow B		Cow C	
	WMT negative[c]	WMT positive[c]	WMT negative	WMT positive	WMT negative	WMT positive
	(molar %)					
4:0	13.35 ± 0.23	12.85 ± 0.18	13.12 ± 0.34	11.24 ± 0.25	15.96 ± 0.44	12.53 ± 0.62
6:0	4.44 ± 0.16	4.81 ± 0.10	4.86 ± 0.28	5.42 ± 0.32	5.66 ± 0.35	6.21 ± 0.18
8:0	3.28 ± 0.17	2.55 ± 0.11	3.09 ± 0.16	2.04 ± 0.24	2.91 ± 0.05	3.10 ± 0.30
10:0	7.59 ± 0.32	5.08 ± 0.31	7.38 ± 0.18	5.15 ± 0.32	6.66 ± 0.49	4.74 ± 0.36
10:1	0.93 ± 0.06	0.96 ± 0.13	0.91 ± 0.13	0.98 ± 0.05	0.87 ± 0.04	1.20 ± 0.06
12:0	6.55 ± 0.35	5.33 ± 0.27	6.43 ± 0.43	4.36 ± 0.40	6.07 ± 0.22	4.39 ± 0.25
14:0	8.76 ± 0.23	8.14 ± 0.46	9.82 ± 0.58	8.21 ± 0.55	8.32 ± 0.15	8.36 ± 0.18
16:0	20.32 ± 0.42	22.61 ± 0.85	18.76 ± 0.91	21.88 ± 0.46	18.46 ± 0.75	22.50 ± 0.22
16:1	1.39 ± 0.02	1.44 ± 0.13	0.87 ± 0.13	1.20 ± 0.05	1.84 ± 0.13	2.64 ± 0.13
18:0	9.30 ± 0.39	9.90 ± 0.42	9.34 ± 0.70	10.90 ± 0.33	6.94 ± 0.49	10.19 ± 0.25
18:1	20.16 ± 0.20	21.35 ± 1.23	21.64 ± 0.75	24.40 ± 0.78	22.42 ± 1.74	20.83 ± 1.08
18:2	3.95 ± 1.11	4.98 ± 0.10	3.80 ± 0.37	4.25 ± 0.06	3.93 ± 0.13	3.33 ± 0.13

TABLE A-8 (Right-hand continuation).

Fatty acid	Free fatty acid composition					
	Cow D		Cow E		Cow F	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(molar %)					
4:0	14.43 ± 0.34	11.60 ± 1.09	13.66 ± 0.30	11.50 ± 0.15	14.04 ± 0.12	11.88 ± 0.33
6:0	4.90 ± 0.31	5.21 ± 0.37	4.50 ± 0.10	4.90 ± 0.29	5.33 ± 0.65	5.81 ± 0.35
8:0	3.03 ± 0.08	2.38 ± 0.15	3.43 ± 0.17	3.02 ± 0.09	3.06 ± 0.25	2.94 ± 0.02
10:0	7.50 ± 0.21	4.73 ± 0.24	7.35 ± 0.32	5.26 ± 0.14	7.27 ± 0.30	5.72 ± 0.12
10:1	0.96 ± 0.04	1.07 ± 0.02	0.86 ± 0.05	1.02 ± 0.03	0.99 ± 0.02	1.07 ± 0.05
12:0	6.27 ± 0.24	5.67 ± 0.21	6.53 ± 0.16	5.87 ± 0.30	6.41 ± 0.33	5.82 ± 0.37
14:0	10.39 ± 0.11	8.26 ± 0.36	10.39 ± 0.10	8.40 ± 0.10	8.53 ± 0.11	8.61 ± 0.07
16:0	17.66 ± 0.94	19.98 ± 0.55	18.22 ± 0.39	18.79 ± 1.53	19.48 ± 0.21	18.27 ± 0.31
16:1	1.55 ± 0.06	2.09 ± 0.20	1.04 ± 0.02	1.33 ± 0.11	2.20 ± 0.07	2.50 ± 0.12
18:0	6.30 ± 0.29	9.19 ± 0.53	7.91 ± 0.48	10.78 ± 0.25	6.86 ± 0.15	7.61 ± 0.18
18:1	23.31 ± 0.89	25.82 ± 0.75	22.09 ± 0.94	24.61 ± 2.41	21.67 ± 0.92	24.82 ± 0.53
18:2	3.70 ± 0.14	4.00 ± 0.27	4.04 ± 0.14	4.53 ± 0.29	4.18 ± 0.10	4.98 ± 0.12

TABLE A-8 (Right-hand continuation).

Fatty acid	Free fatty acid composition					
	Cow G		Cow H		Cow I	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(molar %)					
4:0	13.71 ± 0.08	13.43 ± 0.19	13.56 ± 0.24	11.56 ± 0.09	14.77 ± 0.57	11.42 ± 1.08
6:0	4.68 ± 0.24	4.99 ± 0.59	4.50 ± 0.16	4.96 ± 0.06	4.66 ± 0.11	5.21 ± 0.34
8:0	2.93 ± 0.15	2.41 ± 0.11	3.06 ± 0.26	2.37 ± 0.17	2.94 ± 0.08	2.07 ± 0.38
10:0	7.62 ± 0.37	5.09 ± 0.18	7.27 ± 0.04	5.69 ± 0.13	7.11 ± 0.09	5.35 ± 0.48
10:1	0.88 ± 0.10	1.03 ± 0.04	0.93 ± 0.02	1.19 ± 0.08	0.75 ± 0.13	0.95 ± 0.03
12:0	6.44 ± 0.33	5.39 ± 0.36	6.63 ± 0.14	5.66 ± 0.10	5.98 ± 0.12	5.41 ± 0.05
14:0	11.49 ± 0.16	8.39 ± 0.18	9.54 ± 0.12	8.66 ± 0.04	7.68 ± 0.10	8.17 ± 0.11
16:0	17.79 ± 0.15	19.76 ± 0.52	17.64 ± 0.27	19.09 ± 0.29	19.46 ± 0.21	20.27 ± 0.11
16:1	1.71 ± 0.08	2.04 ± 0.10	2.16 ± 0.09	2.55 ± 0.09	1.18 ± 0.04	1.51 ± 0.09
18:0	8.38 ± 0.23	10.56 ± 0.07	8.51 ± 0.11	10.36 ± 0.10	9.20 ± 0.11	10.50 ± 0.22
18:1	20.22 ± 0.22	21.96 ± 1.08	22.40 ± 0.60	23.88 ± 0.27	22.98 ± 0.60	25.42 ± 0.98
18:2	4.17 ± 0.06	4.97 ± 0.08	3.81 ± 0.04	4.03 ± 0.11	3.38 ± 0.14	3.73 ± 0.07

TABLE A-8 (Right-hand continuation).

Fatty acid	Free fatty acid composition	
	Cow J	
	WMT negative	WMT positive
	(molar %)	
4:0	17.32 ± 0.80	12.00 ± 0.37
6:0	4.54 ± 0.19	5.36 ± 0.18
8:0	2.81 ± 0.09	2.13 ± 0.21
10:0	5.21 ± 0.20	5.00 ± 0.03
10:1	0.81 ± 0.07	0.96 ± 0.05
12:0	6.01 ± 0.19	5.61 ± 0.08
14:0	8.57 ± 0.16	8.57 ± 0.02
16:0	19.09 ± 0.10	19.64 ± 0.25
16:1	1.24 ± 0.01	2.18 ± 0.11
18:0	7.61 ± 0.17	9.66 ± 0.14
18:1	23.08 ± 0.63	24.67 ± 0.63
18:2	3.71 ± 0.05	4.22 ± 0.10

[a]Values represent data from three trials.
[b]No. of carbon atoms:No. of double bonds.
[c]WMT negative (-) < 10 mm.
WMT positive (+) > 20 mm.

TABLE A-9. Standardized weight of the free fatty acid composition of Wisconsin Mastitis Test negative and positive milks.[a]

	Free fatty acid composition					
	Cow A		Cow B		Cow C	
Fatty acid[b]	WMT negative[c]	WMT positive[c]	WMT negative	WMT positive	WMT negative	WMT positive
	(mg/g fat)					
4:0	1.40 ±	2.10 ±	0.63 ± 0.033	1.27 ± 0.033	1.87 ± 0.033	1.77 ± 0.067
6:0	0.60 ±	1.00 ±	0.30 ±	0.83 ± 0.033	0.87 ± 0.033	1.13 ± 0.033
8:0	0.57 ± 0.033	0.67 ± 0.033	0.27 ± 0.033	0.37 ± 0.033	0.60 ±	0.70 ± 0.058
10:0	1.53 ± 0.033	1.60 ± 0.057	0.70 ±	1.17 ± 0.033	1.57 ± 0.067	1.30 ± 0.058
10:1	0.20 ±	0.30 ±	0.10 ±	0.20 ±	0.20 ±	0.30 ±
12:0	1.57 ± 0.067	1.97 ± 0.067	0.73 ± 0.033	1.13 ± 0.033	1.63 ± 0.033	1.40 ± 0.058
14:0	2.37 ± 0.033	3.40 ± 0.058	1.27 ± 0.033	2.43 ± 0.058	2.57 ± 0.033	3.03 ± 0.033
16:0	6.20 ± 0.058	10.57 ± 0.273	2.70 ± 0.058	7.23 ± 0.120	6.37 ± 0.145	9.20 ± 0.058
16:1	0.40 ±	0.63 ± 0.033	0.10 ±	0.40 ±	0.66 ± 0.033	1.07 ± 0.033
18:0	3.13 ± 0.088	5.13 ± 0.120	1.50 ± 0.058	4.00 ± 0.058	2.67 ± 0.120	4.60 ± 0.058
18:1	6.77 ± 0.067	11.00 ± 0.361	3.43 ± 0.088	8.90 ± 0.153	8.53 ± 0.376	9.37 ± 0.260
18:2	1.30 ± 0.200	2.53 ± 0.033	0.57 ± 0.033	1.53 ± 0.033	1.47 ± 0.033	1.47 ± 0.033
Total	26.00 ± 0.058	40.90 ± 0.252	12.33 ± 0.033	29.40 ±	29.00 ± 0.100	35.40 ± 0.100

TABLE A-9 (Right-hand continuation).

Fatty acid	Free fatty acid composition					
	Cow D		Cow E		Cow F	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(mg/g fat)					
4:0	1.43 ± 0.033	1.70 ± 0.116	1.17 ± 0.033	1.40 ±	1.30 ±	1.60 ±
6:0	0.63 ± 0.033	1.03 ± 0.033	0.50 ±	0.77 ± 0.033	0.63 ± 0.033	1.03 ± 0.033
8:0	0.50 ±	0.57 ± 0.033	0.50 ±	0.60 ±	0.47 ± 0.033	0.63 ± 0.033
10:0	1.47 ± 0.033	1.37 ± 0.033	1.23 ± 0.033	1.23 ± 0.033	1.30 ± 0.058	1.50 ±
10:1	0.20 ±	0.30 ±	0.10 ±	0.23 ± 0.033	0.20 ±	0.30 ±
12:0	1.43 ± 0.033	1.83 ± 0.033	1.23 ± 0.033	1.60 ± 0.058	1.33 ± 0.033	1.77 ± 0.067
14:0	2.63 ± 0.033	3.13 ± 0.088	2.30 ±	2.63 ± 0.033	2.00 ±	3.00 ±
16:0	5.03 ± 0.145	8.43 ± 0.120	4.57 ± 0.033	6.63 ± 0.318	5.23 ± 0.033	7.17 ± 0.088
16:1	0.43 ± 0.033	0.90 ± 0.058	0.30 ±	0.47 ± 0.033	0.60 ±	0.97 ± 0.033
18:0	1.97 ± 0.067	4.33 ± 0.120	2.20 ± 0.058	4.23 ± 0.088	2.03 ± 0.033	3.30 ± 0.058
18:1	7.30 ± 0.153	12.03 ± 0.176	6.03 ± 0.145	9.60 ± 0.529	6.40 ± 0.153	10.70 ± 0.200
18:2	1.13 ± 0.033	1.87 ± 0.088	1.13 ± 0.033	1.77 ± 0.067	1.20 ±	2.13 ± 0.033
Total	24.10 ±	37.40 ± 0.153	21.27 ± 0.088	31.27 ± 0.203	22.70 ± 0.058	34.10 ± 0.265

TABLE A-9 (Right-hand continuation).

Fatty acid	Free fatty acid composition					
	Cow G		Cow H		Cow I	
	WMT negative	WMT positive	WMT negative	WMT positive	WMT negative	WMT positive
	(mg/g fat)					
4:0	1.00 ±	1.40 ±	1.40 ±	1.50 ±	1.47 ± 0.033	1.57 ± 0.088
6:0	0.43 ± 0.033	0.70 ± 0.058	0.60 ±	0.83 ± 0.033	0.60 ±	0.97 ± 0.033
8:0	0.33 ± 0.033	0.40 ±	0.53 ± 0.033	0.50 ±	0.50 ±	0.47 ± 0.067
10:0	1.06 ± 0.033	1.07 ± 0.033	1.50 ±	1.43 ± 0.033	1.40 ±	1.43 ± 0.088
10:1	0.10 ±	0.20 ±	0.20 ±	0.30 ±	0.13 ± 0.033	0.27 ± 0.033
12:0	1.06 ± 0.033	1.30 ± 0.058	1.57 ± 0.033	1.67 ± 0.033	1.37 ± 0.033	1.70 ±
14:0	2.13 ± 0.033	2.33 ± 0.033	2.53 ± 0.033	2.90 ±	2.00 ±	2.90 ±
16:0	3.73 ± 0.067	6.10 ± 0.100	5.30 ± 0.058	7.17 ± 0.088	5.70 ± 0.058	8.17 ± 0.033
16:1	0.37 ± 0.033	0.60 ±	0.63 ± 0.033	0.97 ± 0.033	0.30 ±	0.60 ±
18:0	1.97 ± 0.033	3.60 ±	2.83 ± 0.033	4.33 ± 0.033	3.00 ±	4.67 ± 0.067
18:1	4.63 ± 0.033	7.43 ± 0.203	7.40 ± 0.116	9.90 ± 0.058	7.40 ± 0.100	11.30 ± 0.208
18:2	0.97 ± 0.033	1.67 ± 0.033	1.27 ± 0.033	1.67 ± 0.033	1.07 ± 0.033	1.63 ± 0.033
Total	17.77 ± 0.133	26.83 ± 0.033	25.70 ± 0.058	33.17 ± 0.120	24.90 ±	35.77 ± 0.033

TABLE A-9 (Right-hand continuation).

Fatty acid	Free fatty acid composition	
	Cow J	
	WMT negative	WMT positive
	(mg/g fat)	
4:0	1.97 ± 0.067	1.73 ± 0.033
6:0	0.70 ±	1.03 ± 0.033
8:0	0.50 ±	0.47 ± 0.033
10:0	1.13 ± 0.033	1.40 ±
10:1	0.20 ±	0.30 ±
12:0	1.57 ± 0.033	1.83 ± 0.033
14:0	2.53 ± 0.033	3.20 ±
16:0	6.30 ±	8.27 ± 0.088
16:1	0.40 ±	0.93 ± 0.033
18:0	2.83 ± 0.033	4.50 ± 0.058
18:1	8.43 ± 0.120	11.40 ± 0.173
18:2	1.33 ± 0.033	1.93 ± 0.033
Total	27.93 ± 0.088	36.93 ± 0.186

[a]Values represent data from three trials.
[b]No. of carbon atoms:No. of double bonds.
[c]WMT negative (-) < 10 mm.
WMT positive (+) > 20 mm.

TABLE A-10. Composition of membrane preparations from Wisconsin Mastitis Test negative and positive milks.

Trial[a]	WMT value	Total membrane	Lipid	Protein
	(mm)	(g/100g fat)	(% of total membrane)	
A	6	1.14	53.0	47.0
	25	1.03	56.4	43.6
B	7	1.07	52.1	47.9
	28	0.99	61.5	38.5
C	6	1.10	54.9	45.1
	26	0.95	58.2	41.8

[a]Aliquot portions of quarter samples from at least five individual cows were blended on the basis of Wisconsin Mastitis Test values.

TABLE A-11. Composition of delipidated membrane preparations from Wisconsin Mastitis Test negative and positive milks.[a]

Trial[b]	WMT value	Nitrogen[c]	Phosphorus[d]	Cholesterol
	(mm)	(%)	(mg/gm membrane)	
A	6	12.62	5.91	24.1
	25	12.56	5.32	31.0
B	7	11.93	5.59	19.2
	28	10.98	5.35	20.9
C	6	11.84	5.39	32.9
	26	11.09	5.01	32.1

[a]Extraction procedure by Folch et al. (47).

[b]Aliquot portions of quarter samples from at least five individual cows were blended on the basis of Wisconsin Mastitis Test values.

[c]Protein = N x 6.25.

[d]Phospholipids = P x 25.0.

TABLE A-12. Enzyme activity of whole milk and membrane preparations from Wisconsin Mastitis Test negative and positive milks.

Trial[a]	WMT value	Whole milk			Membrane preparation	
		Aldolase[b]	Lipase[c]	Xanthine oxidase[d]	Aldolase[b]	Xanthine oxidase[d]
	(mm)					
A	6	0.048	1.43	10.8	0.007	5.8
		0.053	1.39	11.1	0.008	6.2
	25	0.119	1.71	4.2	0.028	2.9
		0.121	1.76	4.5	0.021	2.8
B	7	0.078	1.63	14.2	0.012	8.6
		0.082	1.58	15.0	0.010	8.4
	28	0.278	1.79	6.3	0.031	4.1
		0.271	1.82	6.4	0.032	4.3
C	6	0.069	1.21	10.9	0.009	7.8
		0.068	1.30	9.8	0.010	7.8
	26	0.150	1.53	5.9	0.027	3.2
		0.142	1.62	6.1	0.022	3.0

[a]Aliquot portions of quarter samples from at least five individual cows were blended on the basis of Wisconsin Mastitis Test values.
[b]Micromoles of 1,6 fructose diphosphate split by 1 mg of protein at 37 C in 1 hr.
[c]Microequivalents of 0.025 N base/min/ml of lipase source.
[d]Units of 0.3×10^{-7} M reduced TTC/mg protein.